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The Cardiovascular and Functional Consequences of Arteriovenous Fistula Formation in Chronic Kidney Disease

by

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For

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Abstract

Background. Native arteriovenous fistula (AVF) is the vascular access of choice and its use c.f. catheters is associated with sustained reduction in mortality. This may be due to factors beyond dialysis catheter associated sepsis. This study aims to investigate the impact of AVF formation on the spectrum of cardiovascular factors that might be important in the pathophysiology of cardiovascular diseases in CKD patients.

Methods. We recruited 43 pre-dialysis patients who underwent AVF formation. Patients were studied two weeks prior to AVF operation, two weeks and three months postoperatively. Haemodynamic variables were measured using pulse wave analysis, carotid femoral pulse wave velocity (CF-PWV) by applanation tonometry and AVF blood flow by Doppler ultrasound. Bioimpedence analysis was performed and patients underwent serial transthoracic echocardiography. Laser Doppler Perfusion Imaging and iontophoresis were used to assess endothelial dependant (ED) and non-endothelial dependant (NED) vasodilatation.

Results. AVF formation was successful in 30/43 patients. Two weeks postoperatively, total peripheral resistance decreased (-17±18%, p=0.001), stroke volume tended to rise (12±30ml, p=0.053) and both heart rate (4±8bpm, p=0.01) and cardiac output (1.1±1.5l/min, p=0.001) increased. Systolic and diastolic blood pressures reduced (-9±18mmHg; -9±10mmHg; ≤ p=0.006). CF-PWV reduced (-1.1±1.5m/sec, p=0.004). Left ventricular ejection fraction (LVEF) increased (6±8%, p<0.001). Patients with successful AVF formation

had a significantly reduced ED vasodilatation in the fistula arm -36±46%, $p<0.001$. Only NED vasodilatation was significantly reduced in the non-fistula arm 23±40%, $p=0.01$. Patients who had unsuccessful AVF operation exhibited no recordable changes.

All the observed haemodynamic changes were largely maintained after 3 months. No change in hydration status/body composition was observed.

AVF formation resulted in a sustained reduction in arterial stiffness and BP as well as an increase in LVEF. Furthermore, there were significant changes in the local and systemic microcirculation. Overall, post AVF adaptations might be characterised as potentially beneficial in these patients and supports the widespread use of native vascular access, including older or cardiovascular compromised individuals.

Declaration

Except where acknowledged, I declare that this thesis is entirely my own work and is based upon research carried out in the Department of Vascular Medicine, University of Nottingham and Department of Renal Medicine, Derby Hospitals NHS Foundation Trust between October 2006 and May 2009.

All measures of myocardial function (echocardiograms) were undertaken by me after appropriate training and assessment by British Society of Echocardiography accredited instructors. To reduce bias, all the echocardiography images were analysed off line by another researcher (Tarik Eldehni) who was completely blinded to the participants' operation outcome.

Biochemical and haematological data were provided by the laboratories at Derby Hospitals NHS Foundation Trust. Separate ELISAs were performed where necessary by laboratory staff in the School of Graduate Entry Medicine and Health, University of Nottingham Medical School at Derby.

All statistical analysis was initially performed by me and then verified prior to publication by an independent statistician, especially in the case of more complex statistical models.

Shvan Korsheed.

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- Of course, I am hugely indebted to the patients who volunteered to take part in my studies. Without their participation, I would not have been able to perform any of the research work described in this thesis.

Publications and abstracts arising from this thesis

Peer reviewed publications

- S Korsheed, CW McIntyre. Higher arteriovenous fistulae blood flows are associated with a lower level of dialysis induced cardiac injury. Hemodialysis International October 2009, 13(4):505-11.
- S Korsheed, MT Eldehni, SG John, RJ Fluck, McIntyre CW. Effects of arteriovenous fistula formation on cardiovascular performance and function. NDT – February 2011, accepted for publication.
- S Korsheed, CW McIntyre. Creation of an arteriovenous fistula is associated with significant local and systemic changes in microvascular function. Under review.

Oral presentation

- S Korsheed, MT Eldehni, SG John, RJ Fluck, McIntyre CW. Effects of arteriovenous fistula formation on cardiovascular performance and function, British renal society, Birmingham 2nd July 2009.

Poster presentations

- S Korsheed, C Kwok Yi, CW McIntyre. Higher arteriovenous fistula blood flows are associated with a lower level of dialysis induced cardiac injury. European Renal Association, Stockholm, Sweden 12th May 2008 (abstract ref: MP303), Renal Association, Glasgow, May 2008 (abstract ref: P104).
- S Korsheed, C Kwok Yi, CW McIntyre. Creation of an arteriovenous fistula is associated with significant changes in systemic cardiovascular performance. Renal Association, Glasgow, May 2008 (abstract ref: P110).
- S Korsheed, CW McIntyre. Creation of an arteriovenous fistula is associated with significant potentially beneficial changes in systemic cardiovascular performance and arterial stiffness. American Society of Nephrology, Philadelphia, USA, 6th Nov 2008 (abstract ref: TH-PO686).
- S Korsheed, RJ Fluck, SG John, CW McIntyre. Creation of an arteriovenous fistula is associated with significant potentially beneficial changes in systemic cardiovascular performance and arterial stiffness. Renal association, Liverpool, April 2009 (abstract ref: P100).
- S Korsheed, RJ Fluck, SG John, CW McIntyre. Creation of an arteriovenous fistula is associated with significant local and systemic changes in microvascular function. Renal association, Liverpool, April 2009 (abstract ref: P151).

- S Korsheed, SG John, C McIntyre. Creation of an arteriovenous fistula is associated with significant potentially beneficial changes in systemic cardiovascular performance and arterial stiffness. World Congress of Nephrology, Milan, Italy 23rd May 2009 (abstract ref: Su521).
- S Korsheed, CW McIntyre. Creation of an arteriovenous fistula is associated with significant potentially beneficial changes in systemic cardiovascular performance and arterial stiffness. American Society of Nephrology, Philadelphia, USA, Oct 2009.

Chapter 1

Introduction

1 Introduction

1.1 Chronic Kidney Disease

1.1.1 Scope of the problem

Chronic Kidney Disease (CKD) is a major public health problem worldwide with increasing incidence and prevalence. World Health Report 2002 and Global Burden of Disease project pointed out that diseases of the kidney and urinary tract contribute to the global burden of diseases, with approximately 850,000 deaths every year and 15,010,167 disability-adjusted life years. They are the 12th cause of death and the 17th cause of disability respectively[1]. A survey of blood samples carried out in the South East of England in 2000/01 found the prevalence of diagnosed CKD to be 5,554 per million population with 84.4% of these patients were unknown to renal services[2]. A systemic review into 26 population based studies reported that the median prevalence of CKD was 7.2% in persons aged 30 years or older and in persons aged 64 years or older prevalence of CKD varied from 23.4% to 35.8%[3].

Associated with this increase in prevalence, is the disproportionate consumption of health care resources. The total coast of care for ESRD patients in the US was around $22.7 billion in 2006[4].

1.1.2 Definition and staging

CKD is defined as the presence of objective kidney damage and/or the presence of glomerular filtration rate of 60 ml/min/1.73m^2 body surface area or less for at least 3 months, irrespective of the underlying aetiology of the kidney damage.

Detection, early initiation of effective therapy and investigation into the epidemiology of CKD was limited by the lack of a uniform terminology. The guidelines proposed from the National Kidney Foundation of the United States through its Kidney Disease Outcomes Quality Initiative (KDOQI) program were introduced to achieve this goal[5]. These guidelines are accepted internationally and they provide a necessary foundation to help to standardize the current medical practice.

The KDOQI working group defined chronic kidney disease in adults as:

- Kidney damage for ≥3 months, as defined by structural or functional abnormalities of the kidney, with or without decreased GFR, manifest by either:
    1. Pathological abnormalities, or
    2. Markers of kidney damage, including abnormalities in the composition of blood, urine or imaging tests.

OR

- Decreased GFR, with or without evidence of kidney damage.

CKD is further subdivided into 5 subgroups according to the degree of severity of eGFR reduction

GFR (mL/min/1.73 m2)	**Stages**	**Description**
≥90	1	Kidney damage with normal or ↑GFR
60-89	2	Kidney damage with mild ↓GFR
30-59	3	Moderate ↓GFR
15-29	4	Severe ↓GFR
< 15	5	End Stage Renal Disease (ESRD)

Table 1.1 Stages of Chronic Kidney Diseases (CKD)

1.2 Cardiovascular Diseases in patients with CKD

Cardiovascular diseases (CVD) are the leading cause of mortality and morbidity in patients with CKD. CKD patients are more likely to die from a CVD than developing kidney failure[6-7]. This increased risk begins in earlier stages of CKD before the onset of renal failure. Patients with CKD not only have a very high prevalence of more conventional cardiovascular risk factors such as diabetes and hypertension but also they are exposed to the complications of the kidney failure such as uraemia related CVD and complications associated with the treatment of the renal failure.

It has been shown in multiple studies that there is evidence of CVD in early stages of CKD. In a cross sectional study, it has been shown that there was a gradual increase in the number of patients who had left ventricular hypertrophy (LVH) as renal function declined with the largest prevalence (45%) when creatinine clearance dropped below 25mL/min[8]. This in contrast to the 20% prevalence of LVH in general population of similar age[9].

It is very well known that patients with CKD have a higher prevalence and incidence of CVD such as ischemic heart diseases and heart failure[10-11]. They are also at higher risk of death after an acute myocardial infarction[12-13]. This is very clearly shown in several epidemiological studies looking into morbidity and mortality in patients with CKD. In the Cardiovascular Health Study, patients with CKD, had heart failure (8%), IHD (26%) and 55% had hypertension. This was in contrast to patients without CKD 13% had heart failure, 3% had IHD and 36% had hypertension. Furthermore, it was shown

that patients with CKD had CVD event rate of 102 per 1000 patient year. This in contrast to subjects without CKD who had CVD event rate of only 44 per 1000 patient year[14]. Further more, the risk of cardiovascular events increases as GFR declines and this has been clearly demonstrated in Atherosclerosis Risk in Communities (ARIC) study which showed that the level of GFR is an independent risk factor for atherosclerotic cardiovascular disease in subjects aged 45 to 64 years.

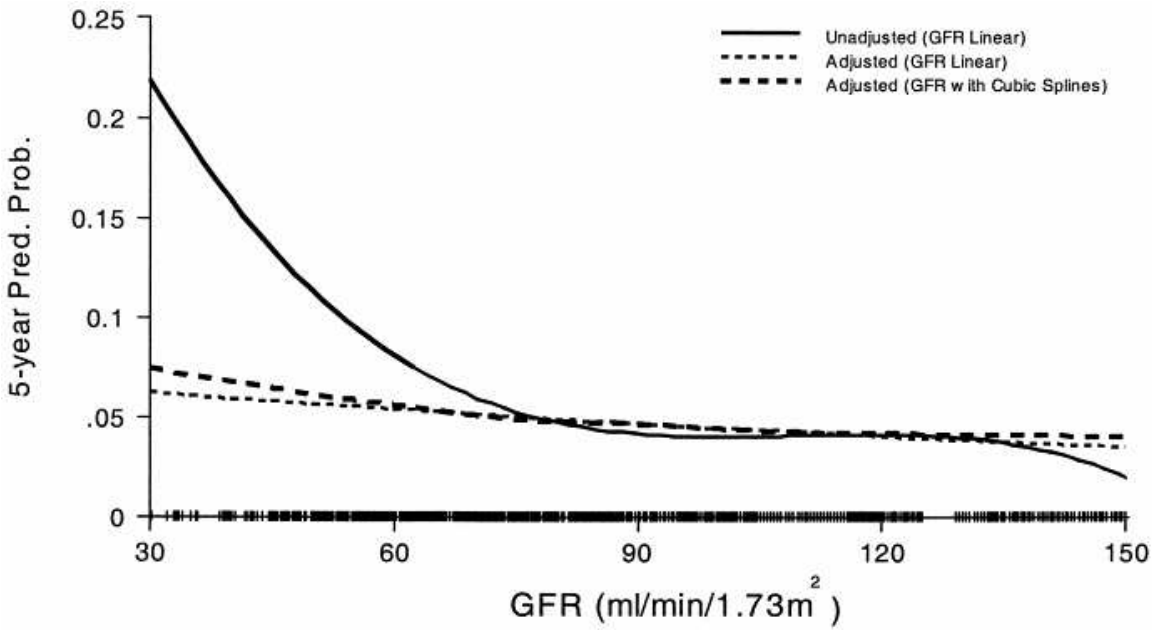

Figure1.1 Smoothed five-year predicted probability of developing atherosclerotic cardiovascular disease by level of glomerular filtration rate (GFR).

Amongst ESRD patients who are on dialysis, CVD is the leading cause of mortality, accounting for up to 45% of deaths[15]. This is also been shown by epidemiological studies reporting increase CV mortality by 10% to 30% in dialysis patients compared to general population[16].

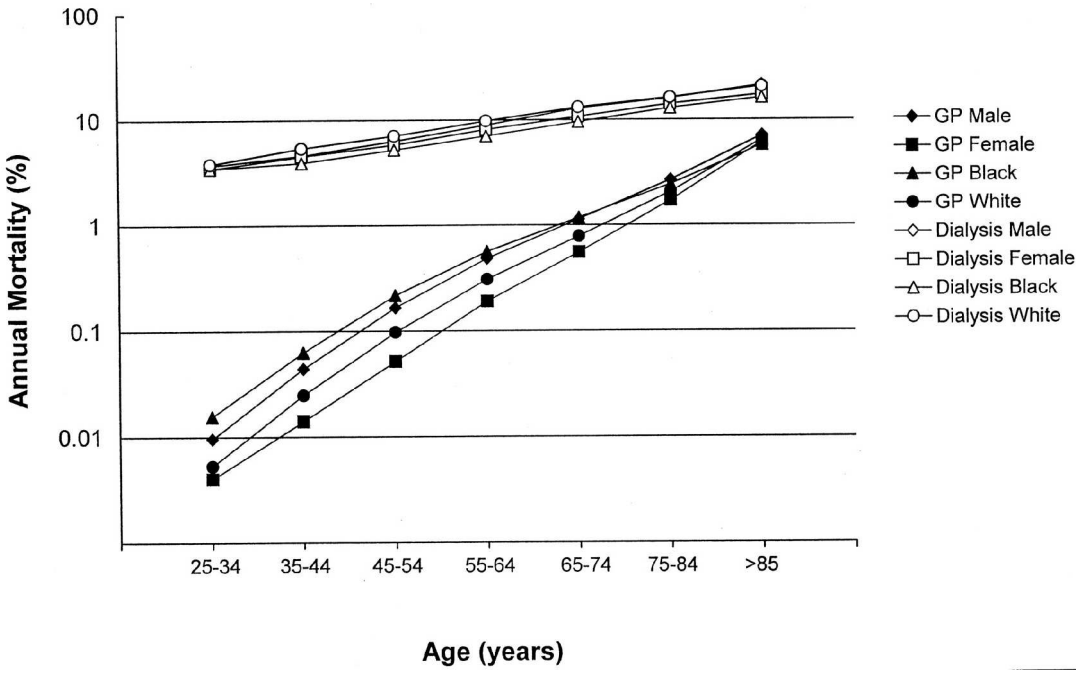

Figure 1.2 Cardiovascular disease mortality by age, race, and gender in the general population and in dialysis patients.

1.3 Risk Factors for CVD in CKD patients

In addition to the traditional risk factors associated with CVD, there are several non-traditional factors which play important role in the pathogenesis and outcome of CVD in CKD patients as shown in the table below.

Traditional risk factors	Non-traditional risk factors
Hypertension	Homocysteinemia
Diabetes	Albuminuria
Smoking	Abnormal calcium/phosphate
Dyslipidaemia	metabolism
Left ventricular hypertrophy	Fluid overload
Inactive life style	Oxidative stress
Male	Inflammation (C-reactive protein)
Age	Malnutrition
Menopause	Thromobogenic factors
Family history	Altered nitric oxide/endothelin balance
	Anaemia
	Vascular calcification
	Elevated lipoprotein A
	Increased asymmetrical dimethyl-arginine(ADMA)

Table 1.2 Risk factors for cardiovascular diseases in patients with CKD.

1.3.1 Traditional CVD risk factors

Blood pressure

As well as being considered an exacerbating factor in CKD, hypertension is one of the major complications in CKD patients. Although the prevalence of hypertension widely varies depending on the nature of the underlying cause of CKD, it increases almost linearly as kidney function falls. Up to 80-90% of patients with different stages of CKD have hypertension[17].

The mechanisms which can contribute to high blood pressure in CKD patients include expansion of the extracellular volume, increased activity of the renin-angiotensine-aldosterone system, abnormal endothelial cell function, abnormal parathyroid hormone level and iatrogenic factors such as erythropoietin stimulating agent administration.

In patients with ESRD on dialysis, hypertension has been associated with adverse CV outcome and it is an independent risk factor for IHD, left ventricular hypertrophy, heart failure and stroke[18] and the overall CV mortality in CKD patients[19]. It has been shown that cardiovascular mortality increased by 2.93 folds in patients with uncontrolled hypertension prior to starting dialysis[20]. In ESRD patients, a mean pre dialysis blood pressure of >98 mmHg was associated with 2.2 fold increase in CV death compared to those with mean blood pressure of <98 mmHg[21]. Controlling hypertension is of paramount importance in slowing the progression of CKD and reducing CV risks in this group of patients[22].

It is important to note that relationship between blood pressure and CV morbidity and mortality in dialysis patients is a complex one. Low blood pressure may indicate heart failure/cardiomyopathy and it predisposes patients to increased episodes of intradialytic hypotension which is associated with increased mortality[23]. In dialysis patients, it has been demonstrated that each 10 mmHg increase in the mean arterial pressure was independently associated with progressive increase in concentric left ventricular hypertrophy and the development of denovo heart failure and ischaemic heart diseases[18]. Paradoxically, the same study showed that lower blood pressure levels were associated with significantly increased mortality,

Diabetes Mellitus

Diabetes is the most common cause of CKD and it account for over 40% of ESRD patients on dialysis. It is generally accepted that 25-40% of patients with either type 1 or 2 diabetes will develop diabetic nephropathy.

The earliest manifestation of diabetic nephropathy is microalbuminuria (>30 mg/24 hours), but recent studies have shown this pattern is changing with patients presenting with increased creatinine and normal urinary albumin levels.

Microalbuminuria on its own is a very significant risk factor for the progression of CKD and the development of other CV diseases[24-25].

Although patients with CKD have significantly higher CV morbidity and mortality compared to general population, the subgroup of CKD patients with diabetes have even increased incidence of peripheral vascular disease, microvascular diseases and ischaemic heart disease. Diabetes is an independent risk factor for IHD, heart failure and all cause mortality in dialysis patients[26]. It has been shown that presence of both diabetes and CKD together increases mortality rate in patients undergoing invasive cardiac investigation and treatment compared to those with CKD patients but without diabetes[27].

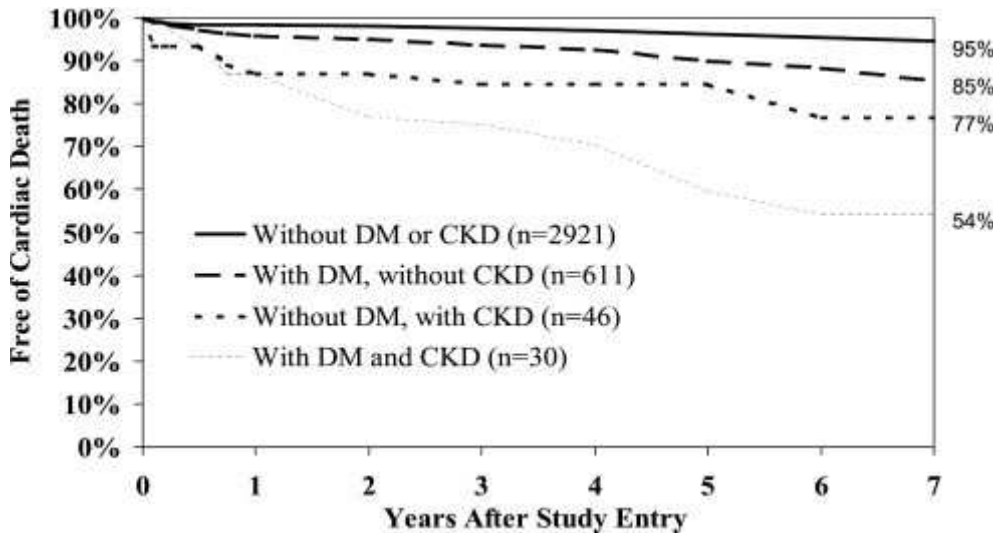

Figure 1.3 Freedom from all cause mortality for patients with CK and diabetes

Left ventricular hypertrophy (LVH)

LVH is a physiological adaptation to a long term increase in myocardial workload. It can happen secondary to increased pressure and/or volume overload. In addition to the conventional factors causing LVH in general population, other factors contributing to LVH in CKD population include increased arterial stiffness, uncontrolled hypertension, increased extracellular volume, anaemia, and presence of AVF.

Pressure overload requires increased intracavity pressure to overcome the increased afterload during systole. This results in hypertrophy. This in turn causes increased myocardial workload and may precipitate myocardial ischaemia in absence of significant coronary artery disease. In addition, volume overload leads to addition of new sacromeres which in longer term results in hypertrophy. This again causes reduction in subendocardial perfusion and subsequent myocardial fibrosis. Eventually, this can end up in dilated cardiomyopathy and systolic dysfunction[28].

In epidemiological studies, LVH is present in up to 50% of CKD pre-dialysis patients and this increases to around 75% in patients on dialysis. There is a negative correlation between LVH on one hand and reduced glomerular filtration rate (GFR), reduced haemoglobin and higher systolic blood pressure on the other hand. LVH is well know to be an independent risk factor for CV morbidity and mortality in dialysis patients[29-31].

Dyslipidaemia

As well as being a risk factor for CVD in CKD population, dyslipidaemia may be a mediator of CKD progression. CKD is associated with profound lipid dysregulation which is different to dylipidaemia in general population. Plasma triglyceride (TGL) concentration is frequently elevated but plasma cholesterol level is usually normal, even reduced or rarely mildly elevated in CKD. The increased level of TGL is associated with high concentration and impaired clearance of very low density lipoprotein (VLDL). There is a similar elevation in plasma chylomicron level due to impaired clearance. Plasma concentration of low density lipoprotein (LDL) is normal and only mildly elevated in CKD. High density lipoprotein concentration is almost always reduced inpatients with CKD.

Due to this unusual form of dyslipidaemia, it has been reported that the relationship between cholesterol level and mortality in CKD patients is more complex than general population. Low cholesterol levels were associated with increased mortality in dialysis patients[32-34] . It is believed that increased all cause and cardiovascular mortality in dialysis patients with low cholesterol is due to the association between hypocholestrolaemia and increased inflammation/malnutrition[34].

1.3.2 Non traditional CVD risk factors

Homocysteinemia

Homocysteine (Hcy) levels increase as GFR declines due to reduced clearance of Hyc. Hyperhomocystienaemia is much more common in dialysis patients and it is believed that up to 80% of patients in ESRD have elvated level of Hcy. Higher level of Hcy has been implicated as a risk factor for myocardial infarction and stroke in ESRD patients. Few studies have shown that homocysteinemia is an independent risk factor for CVD mortality in dialysis patients[35].

Not all studies have shown that hyperhomocyteinaemia to be an independent risk factor for CV diseases in dialysis patients. Furthermore, Hyc levels has been associated with nutritional status and in subjects with nutritional deficiencies, low Hcy levels are associated with a worst outcome[37], therefore nutritional status, albumin level and presence of other co-morbidities should be taken into consideration when evaluating Hcy level as a risk factor for CV diseases in CKD patients.

Lowering Hcy levels has not been constantly associated with a favourable outcome. Indeed, a more recent trial demonstrated that lowering homocystiene levels with a combination of folic acid, vitamin B6 and B12 did not reduce the risk of major cardiovascular events in patients with vascular diseases[36].

Inflammation and Oxidative stress

Renal failure causes changes in plasma components and endothelial structure and function that favour vascular injury, which may play a role as a trigger for Inflammatory response[38-40]. Up to 50% of CKD patients have elevated serum levels of inflammatory markers such as C-reactive protein, fibrinogen, interleukin-6, and tumor necrosis factor[41-44].

Mechanisms are unclear but increased inflammatory mediators have been attributed to increased oxidative stress, advanced glycation end products (AGE), and other agents normally cleared by the kidney.

The mechanisms causing increased oxidative stress in CKD is not completely understood. Increased production of radical oxygen species combined with reduced clearance in addition to an impaired antioxidant defence system might all contribute. Additionally, CKD usually coexists with other diseases (diabetes and hypertension) that are known to be causing oxidative stress.

Several mechanisms have been proposed as the cause of oxidative stress in uraemia. These include activation of reduced nicotinamide adenine dinucleotide oxidase, xanthine oxidase and myeloperoxidase [40 45].

Kidney plays an important role in the clearance of AGE. There is a growing body of evidence suggesting that increased levels of AGE is associated with an increase in the proinflammatory and oxidative stress status, endothelial dysfunction[46-47] and arterial stiffness.[48-49]

Endothelial Dysfunction, Nitric Oxide bioavailability, and Asymmetric dimethyl arginine (ADMA)

Endothelial dysfunction is recognised as one of the initial mechanisms that lead to atherosclerosis. Endothelial dysfunction, which occurs in both large and small arteries, is present in renal disease[50]. Microalbuminuria, a marker of glomerular hyperfiltration, has been correlated with and may be a manifestation of impaired endothelial function[51].

Reduced bioavailability of nitric oxide (NO) appears to be one of the main factors involved in chronic renal failure–associated endothelial dysfunction. ADMA is a competitive inhibitor of NO synthase and has been implicated as the potential link between endothelial dysfunction and CVD in CKD. ADMA is primarily cleared by the kidneys and it accumulates in renal impairment. In vitro, ADMA inhibits NO generation, and in humans it reduces forearm blood flow and cardiac output and increases systemic vascular resistance and blood pressure[52].

Plasma concentrations of ADMA are increased in association with endothelial dysfunction and/or reduced NO production, particularly in renal failure[53]. Elevated plasma concentrations of ADMA are associated not only with endothelial dysfunction and atherosclerosis[54] but predict mortality and CV complications in CKD and end stage renal failure[55].

Arterial stiffness

There is an increasing awareness that abnormal large artery function plays an important role in the pathogenesis of CVD. Arterial walls alter their structure and function in response to atherogenic and atherosclerotic factors, as well as changes in the haemodynamic burden. As a result, the structural changes can include activation and proliferation of smooth muscle cells and rearrangement of cellular elements and extracellular matrix of the vessel wall[56]. This disruption of the architecture with the increase of collagen and loss of elastic fibres results in a reduction in arterial compliance and an increase in arterial stiffness.

Increased arterial stiffness, with an associated increase in the amplitudes of the forward and reflected pressure waves, is a major determinant of increased systolic and pulse pressure with advancing age. Increased pulse pressure and aortic augmentation index, as indirect measures of arterial stiffness, and carotid-femoral pulse wave velocity (CF-PWV), a more direct measure of stiffness, are associated with adverse clinical events.

Pressure wave reflection serves two beneficial purposes. When normally timed, the reflected wave returns to aorta in diastole and therefore enhances diastolic perfusion pressure in the coronary circulation. Partial wave reflection also returns a portion of the pulsatile energy content of the wave form to the central aorta where it is dissipated by viscous damping. Thus, wave reflection limits transmission of pulsatile energy into the periphery where it might otherwise damage the microcirculation. Thus the loss of this protective

function of wave reflection could contribute to the pathogenesis of a growing spectrum of cardiovascular and non cardiovascular complications.

It has been shown that there is a positive association between GFR and arterial stiffness[57]. It was also demonstrated that there is a step wise increase in arterial stiffness as CKD stages progresses from stage1 to stage5 [58].

It has also been demonstrated that aortic pulse wave velocity and arterial wave reflections predict the extent and severity of coronary artery disease in chronic kidney disease patients[59].

In addition to being a likely consequence of CKD, increased aortic stiffness determined by measurement of aortic PWV was shown to be a strong independent predictor of all-cause and mainly cardiovascular mortality in dialysis patients[60](see figure 1.4). Furthermore, same study demonstrated that an increase in the PWV by 1 m/s resulted in an increase in the all cause mortality adjusted odd ratio by 1.39.

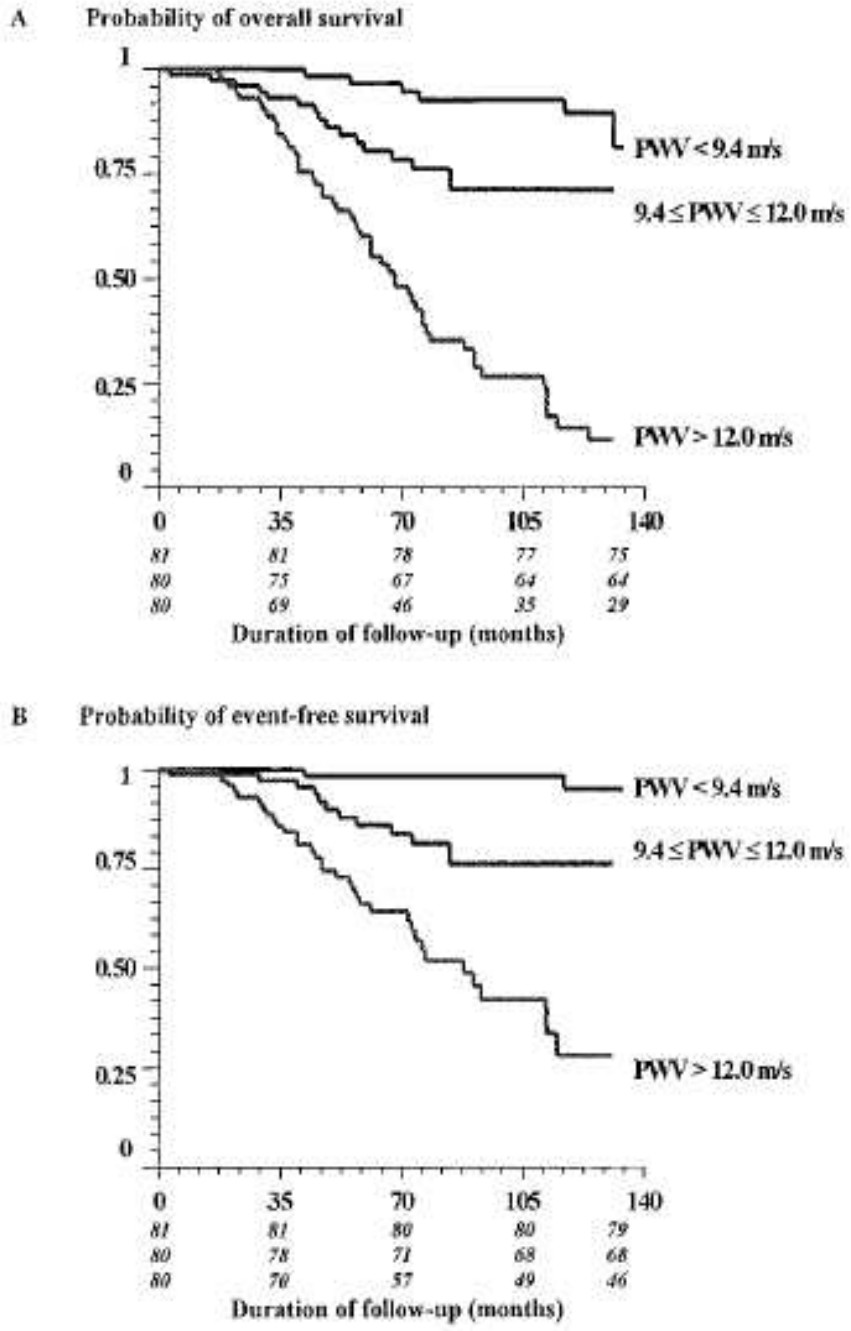

Figure 1.4 A)overall and B)cardiovascular mortality according to PWV divided into tertiles

1.4 Haemodialysis access

Maintaining a functional haemodialysis (HD) access is one of the important challenges nephrologists face today. Vascular access failure is associated with significant morbidity and fistula dysfunction is the most common reason for recurrent hospitalisation and need for secondary intervention [61]. This however represents only the tip of the iceberg. Vascular access complications account for 16–25% of hospital admissions in dialysis patients and depending on the type of vascular access in use, is correlated with overall and cause-specific mortality [62]. There is significant cost associated with vascular access failure; 8000 US dollars per patient at risk per year in the United States, ~15% of Medicare's expenditure for end-stage renal disease [63]. Thus, long term functioning of vascular access is of crucial importance in haemodialysis patients.

1.5 Arteriovenous fistula for haemodialysis

1.5.1 Definition

An AVF is a surgically placed shunt whereby an artery (a high pressure vessel) is anastomosed to a vein (low pressure vessel), forming a high flow low resistance system allowing blood flow from the artery to the vein.

As flowing blood seeks the path of least resistance, the fistula acts effectively as a short-circuit between the arterial circulation and the venous circulation. Over time the created fistula matures; opening the fistula reducing peripheral resistance and dramatically increases flow through the proximal artery [64]. To accommodate the increased volume of blood flow through the fistula, without increasing the shear wall stress excessively, there is usually a widening of the fistula circuit. As the fistula matures, the proximal vein becomes more prominent, more thick walled and it is this that facilitates successful, repetitive percutaneous venepuncture for haemodialysis.

Blood flowing through a fistula is determined by the sum of flow resistance of the arterial system, the anastomosis itself and the flow resistance of the venous system. Blood flow will be in the direction of high to low pressure, therefore flow in all arterial limbs will be towards the anastamosis and in venous limbs away from the anastomosis.

Adequate fistula maturation, i.e. sufficient dilatation and arterialisation, is a prerequisite for repeated cannulation for haemodialysis treatment [65]. Factors

such as age, sex, blood pressure and associated illnesses are likely involved in AVF maturation [66-67].

1.5.2 History of haemodialysis/ AVF development

Dialysis was first described by Thomas Graham in 1854. He described separating substances through a semi-permeable membrane[68]. While a significant amount of research was conducted into artificial membranes between 1880-1913, it wasn't until 1914 Abel et al. tested the first efficient dialysis system at Johns Hopkins University School of Medicine[69]. The first human haemodialysis was performed in a uraemic patient by Haas in 1924 at the University of Giessen in Germany[70]. To obtain access, he used a glass cannula to cannulate radial artery and returned the blood to cubital vein. Initially he used hirudin for anticoagulation which was replaced later with heparin due to side effects.

This was followed by significant improvement in dialysers and membrane designs in 1940's and 50's. Although haemodialysis technology continued to develop, the technique of obtaining access did not evolve equally alongside. The cannulation technique was proving to be even less effective and was prone to complications. In 1960 the first external AV shunt was invented by Scribner and colleagues, using a rigid Teflon tube held over a stainless steel plate with each end cannulating an artery and vein respectively. They were more successful in providing long term intermittent haemodialysis sessions than previous methods. Previous publications quoted mean arterial cannula

survival of 7.8 months and mean venous cannula survival of 7.2 months[71]. Complications associated with these shunts included clotting, bleeding, infection of the cutaneous cannula and subsequent systemic sepsis.

It was not until the surgeons Cimino and Brescia in 1966 created the first subcutaneous shunt by performing a side to side anastomosis between radial artery and cephalic vein[72]. The current AVF is still based on the same design with one modification. Currently an end vein to side artery anastomosis is more in practice as it is associated with fewer complications than the original Cimino fistula.

1.5.3 Types of Vascular access

1.5.3.1 Arteriovenous Fistula

Why Native AVF is the best dialysis access?

Native AVF is the vascular access of first choice[73]. The most recent and valuable data is obtained from Dialysis Outcomes and Practice Patterns Study (DOPPS) which is a prospective cohort study of haemodialysis practices based on the collection of observational longitudinal data for a random sample of patients from dialysis units in 12 countries. It concluded that when used as a patient's first access, AVF survival was superior to grafts regarding time to first failure. It is well known from clinical practice that AVF

have longer periods of usefulness, higher flow rates and lower associated complication rates in comparison to other forms of access.

Recent guidelines published by the American National Kidney Foundation-Kidney Disease Outcomes Quality Initiative (KDOQI) recommend that at least 50% of new haemodialysis patients have a primary AVF, leading to better patency rates and lower access-related costs [74]. The use of definitive vascular access in HD patients, rather than tunnelled central venous catheters, is associated with sustained reduction in mortality over at least three years[75]. AVF has been proven repeatedly to have the lowest risk of infection and thrombosis compared to other forms of access.

Anatomical locations

Autogenous upper arm AVF can be created in arm or forearm. The usual practice is to start as distally as possible in the non dominant arm and move proximally. Doppler ultrasound has been used in vascular mapping for assessing anatomical (diameter) and physiological (flow rate) parameters of the blood vessels preoperatively. The exclusion criteria are widely variable between different centres; however; generally speaking a vein diameter of less than 2.5-3 mm and abnormal arterial flow pattern or reduced flow are contraindications for AVF formation. The first anatomic location to be initially considered for anastomosis is radial artery-cephalic vein. Other less commonly used anastomosis are radial artery-basilic vein and ulnar artery-basilic vein. However, these are more technically challenging to construct, needling requires much more skill and it is less comfortable for the patient to

stay in a specific posture for the duration of the dialysis treatment. In upper arm, brachocephalic AVF is most commonly used as it is more straightforward to construct. Brachiobasilic AVF formation is more challenging for the surgeon as it requires dissection and mobilisation of basilic vein to make it more superficial for future needling. However, recent studies have shown barchiobasilic AVF to have better outcomes and its use are becoming more popular[76].

Disadvantages of AVF

Although AVF formation is universally the choice of vascular access for all the reasons previously discussed, there are disadvantages. Firstly, AVF is required to undergo a maturation process of 2-3 months. An AVF may fail to mature (failure of outflow vein to enlarge sufficiently to allow for repeated cannulation), resulting in the need for a catheter to initiate dialysis. A non functional AVF is defined as the inability to use the AVF for 2- needle haemodialysis within 8 weeks of surgery [65]. It is recognised that AVF fail to mature in at least one third of cases [77]. Failure of maturation can be attributed to inadequate arterial inflow, outflow vein stenosis, poor surgical anastomosis and development of collateral veins that delay maturation by siphoning flow [78]. The fistula should be examined approximately 4 weeks after creation and referred for imaging and correction of identified lesions if not maturing by 6 weeks. Most non-maturing fistulae have identifiable lesions that can be corrected by percutaneous techniques. In a series of 100 AV fistulae with early failure, 78% had a venous stenosis, 38% anastomotic

stenosis and 46% accessory veins diverting blood and preventing adequate maturation. After percutaneous treatment, 92% became usable for hemodialysis and 84% remained functional at 3 months, 72% at 6 months and 68% at 12 months[79].

For AVF s that thrombose within few days to weeks of their creation, salvage is not usually attempted due to low success rates.

Complications of AVF

AVF can dilate and become aneurysmal due to damage and weakening in the wall following repeated cannulation. Pseudoaneurysm may form following extravasation of blood after dialysis. These carry the risk of rupture and fistula failure.

AVF can also be complicated by thrombosis, infection and haemorrhage. Thrombosis is a leading cause of vascular access failure and is the outcome of stenosis caused by progressive neointimal hyperplasia in the venous outflow system [80].

Dialysis associated steal syndrome is also a recognised complication of fistula formation. This is more common in patients with vascular diseases, diabetes and in elderly patients. It is defined as a clinical condition caused by arterial insufficiency distal to the dialysis access owing to diversion of blood into the fistula. The aetiology is iatrogenic and the fistula usually requires banding to reduce flow or to be tied off completely in more severe cases.

Venous hypertension can occur with all types of AVF and grafts. The most severe form occurs in combination with central vein stenosis secondary to

previous central venous catheter insertion. This can usually be treated with angioplasty and stenting.

High output cardiac failure has been associated with high AVF flow. It is believed to be more common with high flow upper arm AVF and grafts. The literature mostly describes improvement in cardiac failure symptoms after AVF closure[81-82] in those patients as having been selected with worsening problems of congestive syndrome. In other case studies, it has been reported that decrease in the cardiac output with the shunt closure that is less than the blood flow in the unclamped fistula is an indication of high output cardiac status[83]. However, this has not been validated.

Local effects of AVF formation

AVF formation has direct effects on multiple vessels, not only the artery and the vein used to form the AVF. Blood flow rate of fistula is expected increase gradually and can vary from 300 ml/min to even more than 2000 ml/min. Chronic changes in large artery blood flow rates induce adjustments in arterial diameter [84]. Blood flow regulates vessel diameter through changes in shear wall stress [84]. Comparison of flow in the radial artery with AVF and flow in the contralateral arm (as control) showed a six-fold increase in blood flow on the fistula side compared to the control [84]. This was associated with a 1.4-fold increase in the internal radial artery diameter. There was also evidence of structural remodelling, (intima-media thickening) of the arterial wall. Kim *et al* showed that increased radial artery intima-media thickness is closely

associated with early failure of radiocephalic AVF in haemodialysis patients [85].

Endothelial dysfunction has been linked with the initiation and acceleration of the atherosclerotic process [86]. Ene-Iordache *et al* suggested that the radial artery dilates in such a way as to maintain the peak wall shear stress constant and that endothelial cells sense this maximum rather than time-averaged wall shear stress, greatening the endothelial response [87].

AVF creation and effects on cardiac function

One of the first studies done on animals was by Guyton et al who demonstrated up to 82% compensatory increase in cardiac output in response to opening an artificial arterovenous fistula[88].

The literature on the systemic effects of AVF formation in human being is limited. All the studies done prospectively were limited by the number of the patients studied, the frequency and duration of the follow up and the techniques used to assess cardiovascular structure and function.

Nevertheless, they do provide some insight into the systemic changes associated with AVF formation. It is now more evident that haemodialysis and AVF formation affect cardiac function. Six weeks after AVF formation Sandhu et al demonstrated that cardiac index and stroke volume index had increased and there was a significant effect on systolic and mean systemic arterial pressure[89]. . A study by Savage et al confirmed the possibility that the creation of an AVF in CRF patients may predispose to myocardial ischemia

caused by an adverse imbalance between subendocardial oxygen supply and increased oxygen demand [90].

Iwashima et al showed that creation of an AVF has significant effects on cardiac systolic and diastolic performance and detected brain natriuretic peptide (BNP) release stimulated by LV diastolic dysfunction [91].

1.5.3.2 Arteriovenous Grafts (AVG)

Grafts are used as an alternative when AVF formation is unfeasible (e.g. vascular mapping reveals vessels not suitable for AVF). They allow selection of arteries and veins to be joined together. They can be made of synthetic material such as polytetrafluoroethylene (PTFE) or biological material (biograft). Depending on the vessels selected, different anatomical variations can be used to form a graft. For example, forearm loop graft is formed between brachial artery and cephalic vein and straight forearm graft is formed between radial artery and cephalic vein. Lower extremities can also be used for placing straight or loop grafts. Other unusual and rarely practiced grafts are necklace and grafts between lower and upper body blood vessels.

However, the risks associated with AVG formation, complications and failure rates are much worse than for AVF. In a study it was shown that patients with an AV fistula and who were older than 65 years had a risk of access failure that was 24% lower than similar patients with an AVG[92]. The Canadian haemodialysis morbidity study demonstrated a 71% lower failure rate of AVF compared to AVG after adjustment for age, gender and comorbid

conditions[93]. A recent series demonstrated that 77% of AVG required surgical or radiological intervention to maintain patency at one year. Additionally, 45% of AVG permanently failed, requiring replacement during this interval [94].

1.5.3.3 Venous catheters

Cuffed venous catheters (CVC) are recommended to provide only temporary vascular access, with the advantages of ease of insertion and immediate use. Short term use only is advised because of the high rate of complications associated with CVC use.

Infection is the most serious common complication associated with using CVC. Using these catheters expose patients to enhanced risk of catheter related sepsis including bacteraemia and metastatic infection. In the HEMO study it was demonstrated that catheters were present in 32% of all study patients admitted with access-related infection, even though catheters represented only 7.6% of vascular accesses in the study[95]. The 11th annual report from the UK renal registry highlights this fact even further. It reported that 4.2% of MRSA bacteraemia in the UK happened in dialysis patients and of those 70% were dialyzing through a central venous catheter, the majority of which was a tunnelled line (59.8%). The relative risk of MRSA bacteraemia was about 100 fold higher for a dialysis patient in comparison to the general population and 8 fold higher for a patient using a catheter in comparison to a fistula[96]. Actively avoiding infection in dialysis patients is very important as

mortality secondary to sepsis in this cohort of patients is 100 to 300 fold higher than in general population[97].

Vascular catheter malfunction secondary to peri-catheter fibrin sheath and thrombus formation is common and often limits duration of catheter utility [98]. They concluded that most infection-related hospitalizations were not attributed to vascular access. However, the frequency of access-related, infection-related hospitalizations was disproportionately higher among patients with catheters compared with other forms of access.

Another serious and common complication associated with using central venous catheters is subclavian vein stenosis. Almost 25% of dialysis patients with dysfunctional fistulae were found to have subclavian stenosis in one study, all with a history of previous subclavian vein catheterisation[99]. Another study demonstrated that up to 42% of patients undergoing tunnelled catheter insertion, had some degree of unexpected stenosis and/or angulation of the central veins and it recommended using venography immediately prior to the catheter insertion to detect unexpected and clinically significant anatomical abnormalities particularly in those patients with a history of previous tunnelled catheter insertion[100]. Overall, AVF is recommended over catheter use long term for haemodialysis because of better dialysis outcomes and lower associated complication rates.

Chapter 2

Thesis Aims

2 Thesis Aim

2.1 Hypothesis

This thesis has been planned to test the following hypothesis:

Formation of an AVF and the subsequent degree of flow through it is capable of inducing significant microvascular, macrovascular and myocardial, structural and functional changes.

2.2 Research questions

To test this hypothesis, the following interrelated research questions will be addressed:

Primary End Point

- Is AVF formation associated with change in arterial stiffness? If so, how?

Secondary End Points

- Are there any other changes in systemic haemodynamics that can be attributed directly to AVF creation?
- What are the changes in flow of AVF and local circulation over time?
- Are there changes in cardiac function that can be associated with AVF formation or fistula flow?
- Can we identify increased cardiovascular risk by assessing and monitoring fistula flow or microcirculatory function?

Chapter 3

Methodology

3 Methodology

All methods are dealt with in detail in this chapter and then referred back to for reference in the relevant results sections. Certain methods and techniques were used in more than one study outlined below.

3.1 Study design

A research protocol was created for a single centre, prospective, observational study to take place over a 18 months period. The described study has been initiated and conducted in compliance with the written protocol, the Research Governance Framework, the International Conference of Harmonisation, Good Clinical Practice and all applicable Derby Hospitals NHS Foundation Trust Research Office requirements.

Ethical approval was sought and granted for all aspects of the research detailed in this thesis. Application was made to the Central Office of Research Ethics Committees (COREC). Single centre research ethics committee applications were made for the study. Site specific assessments were undertaken and submitted. Approval from the Derby Hospitals NHS Foundation Trust Research and Development Department was also sought using the centralised research and development application form that was integrated into the COREC application system. Joint sponsorship was granted between the Hospital Trust and the University of Nottingham to allow for the collection, analysis and storage of data and pathological samples (plasma and serum) within both institutions.

3.2 Subject selection

Approximately 100-130 fistula formation procedures are performed each year in Derby hospitals. We planned to study up to 50 patients over a period of 18 months. These included adults of any age, with CKD stage 4 and 5 disease who require would haemodialysis. Study subjects were identified from renal outpatient clinics at Derby City General Hospital. Patients required a fistula in Derby were listed as part of routine work-up for renal replacement therapy (RRT). Thus identifying subjects for recruitment was fairly straightforward. Subjects were provided with verbal and written information regarding the research study. A full week was given for patients to consider entry into the study and review the consent form. After one week they were then invited to attend for a recruitment visit. At this visit patients' informed consent was taken. There was no change or reduction to the standard care provided to study patients as they progress to RRT.

Exclusion criteria were kept to a minimum in order to gain sufficient data and reveal as fully as possible, the effects of AVF formation.

Exclusion criteria

- Age <18
- Recent acute illness that required treatment, without full recovery.
- Medically unstable patient.
- Cardiac transplantation.

Inclusion criteria

- Male and female patients aged >18
- Any patient with CKD 4-5 who is clinically stable, including;
    - AVF pending,
    - any cause of renal failure,
    - any co-morbid factors.

3.3 Subject withdrawal

Participants wishing to withdraw were free to do so at any time. This caused no detriment to their usual standard of care.

3.4 Outline of research study

This study was powered to the measurement of arterial stiffness by means of pulse wave velocity. A minimum of 29 patients were required for statistical validity (see statistical consideration - section 3.31). Ethical approval was given for recruitment of up to 50 patients into the study. Renal patients at CKD 4/5 (approaching end stage renal failure), prior to their fistula formation procedures, were being approached.

Patients were to be assessed before and after fistula formation. Assessment included cardiac function, full vascular assessment, centrally and of the fistula limb locally. Distal systemic effects in the opposite limb were also evaluated. Well-established techniques were used to assess these and relevant data are collected. Comparison of the data pre and post fistula formation was made and any changes that result was highlighted. Repeated assessments took place at stages as outlined in table 3.1 using the same techniques each time. Baseline assessments could then be compared to the results at each stage.

3.4.1 Pre assessment

Assessment prior to fistula formation with echocardiography provides a baseline for cardiovascular performance of our study patients. Vascular surgeons require thorough pre-fistula assessment and vascular mapping before surgery to ensure feasibility for AVF. USS Doppler training undertaken over several months has allowed me to perform the relevant vascular

assessment on the study patients. Inter-observer error is also reduced by performing all vascular mapping for the study.

Data relating to macrovascular and microvascular function was gathered at this stage using a range of non invasive, tried and tested techniques as described. They include laser Doppler imaging, pulse wave analysis with augmentation index, pulse wave velocity and finometry. Bioimpedance was also used to measure total body water and body composition.

3.4.2 Newly formed AVF

Two weeks post AVF surgery was the first point of re-assessment (this is pre-dialysis). This includes flow and vascular analysis using same techniques; pulse wave analysis and velocity, finometry, ultrasound Doppler and laser Doppler imaging and echocardiography. Bioimpedance is re-measured.

3.4.3 Mature AVF

A third assessment took place when the fistulae had matured, this normally occurs by 9 weeks post surgery. Dialysis may have commenced at this time. All analyses using the same techniques were repeated. Cardiovascular assessment with Echocardiography was repeated.

Any complications that occurred throughout the study with dialysis or fistulae of patients (thrombotic occlusion, infection, failure to mature etc) were recorded. Those patients that suffered AVF failure, as a result, exit the main

study but monitoring continued. Re-entry to the study occurred if a new AVF was to be created.

3.5 Data collection

1. Initial data collection, pre AVF:
    - Demographic information.
    - Cause of renal disease.
    - Dates of planned fistula and start date for haemodialysis.
    - Past medical history, including vascular disease and all relevant risk factors.
    - Full drug history, including any changes prior to and throughout study.
    - Details of previous cardiac investigations (echocardiograms, LVEF, stress tests, angiography)
    - Baseline routine blood results (full blood count, electrolytes, coagulation).
    - Vascular mapping data.
    - Arterial stiffness measuring pulse wave velocity and augmentation index.
    - Haemodynamic function and baroreflex sensitivity.
    - Cardiac functional assessment with 2-dimensional echocardiography.
    - Microcirculation and endothelial function using laser Doppler imaging plus iontophoresis.

2. Data collection 2/52, post AVF surgery:

- Dialysis details if initiated and QA calculation.
- Routine blood results (full blood count, electrolytes, coagulation).
- Pulse wave velocity and augmentation index.
- Haemodynamic function and baroreflex sensitivity.
- Vascular assessment with laser Doppler (LDI).
- USS Doppler with colour flow.
- Cardiac functional assessment with 2-dimensional echocardiography.
- Body composition using BIA.

3. Data collection, 9/52, mature AVF:

- Dialysis details if initiated- (time at ESRF, time at initiation RRT, monthly kt/v, mode of access including changes, complications if any, blood flow and QA, average interdialytic gain, use of anticoagulant).
- Routine blood results.
- Pulse wave velocity and augmentation index.
- Haemodynamic function and baroreflex sensitivity.
- Cardiac functional assessment with 2-dimensional echocardiography.
- Laser Doppler imaging.
- USS Doppler with colour flow.
- BIA.

Additional measurements at each stage

- Regular review of drug history with documentation of any changes.
- Details of any vascular events, complications or access problems and the treatment given.
- Any events or changes to regular dialysis program.

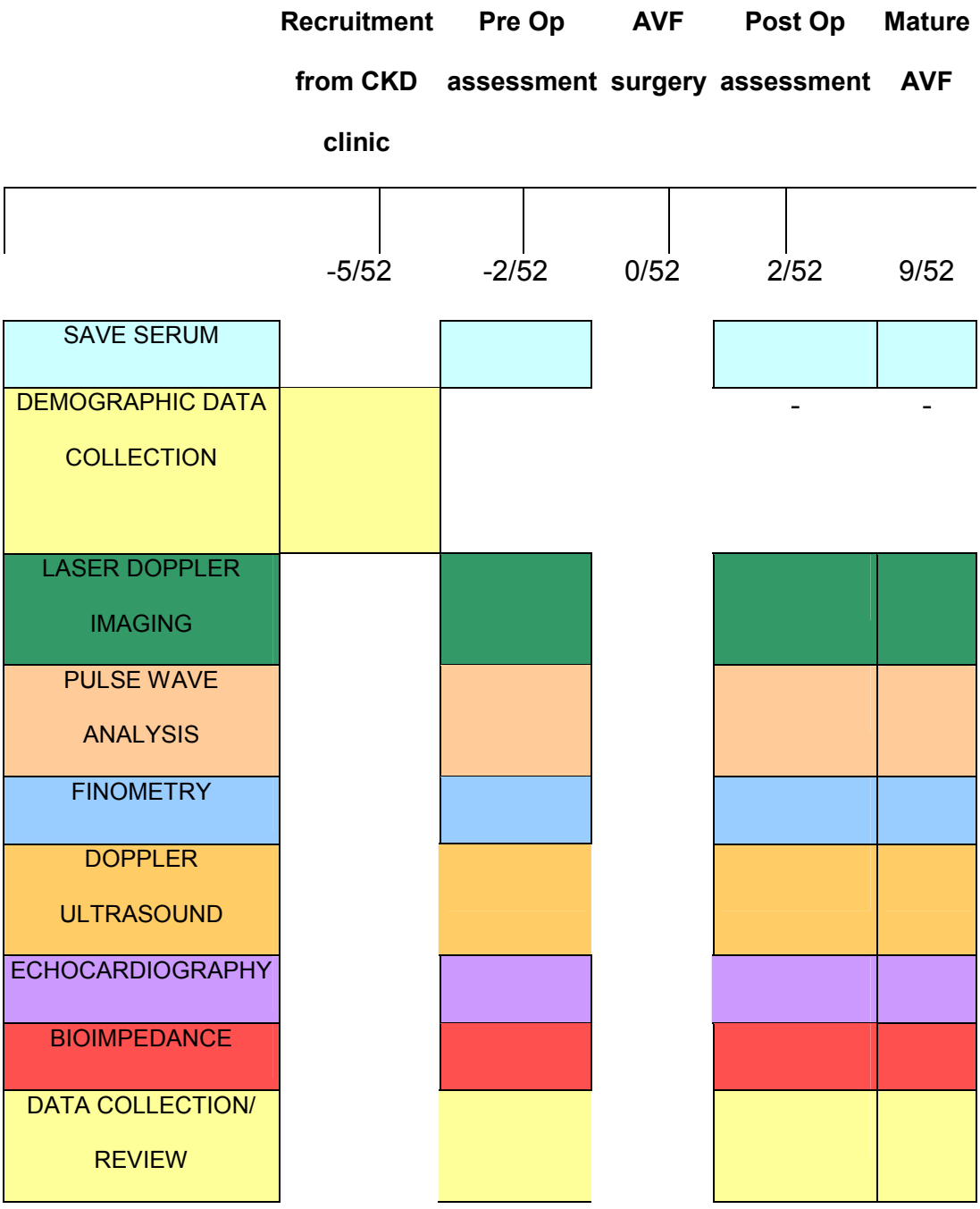

Table 3.1 Outline of the study

3.6 Assessment of Cardiovascular Function

3.6.1 Echocardiography

2-dimensional echocardiography is used to assess cardiac function pre and post fistula formation and results compared. Commercially available equipment, Vivid 3® ultrasound machine with a dedicated cardiac probe ((1.5-3.6 MHz 3S probe, GE medical systems, Sonigen, Germany) is used. Each patient is to have three echocardiogram assessments over the study period. Any prior echocardiograms of study patients provided additional information only. Measurement aims are simplified to provide only the data we require, this includes:

1) Regional left ventricular function assessed by fractional shortening in each LV region at rest.
2) Global LV ejection fraction.

Comment on global LV systolic function. Standard apical 2-chamber and 4-chamber views (to visualise the LV endocardial border in 2 planes at 90^0 to each other) are performed and recorded onto DVD for off-line analysis. Ejection fraction (EF) is calculated using LV volumes at end systole and end diastole from M-mode echocardiography images taken, according to the recommendations of the American Society of Echocardiography[101]. All measurements with the patients positioned in the left lateral position.

3.7 Assessment of arterial stiffness

3.7.1 Pulse wave velocity

PWV is a well-recognised technique for obtaining a measure of arterial stiffness between two locations in the arterial tree. The velocity of the pulse wave along an artery is dependent on the stiffness of that artery. Arterial stiffness, as mentioned before, is directly associated with increased risk of cardiovascular disease. Aortic pulse wave velocity measurement has been shown to be a strong independent predictor of cardiovascular and all-cause mortality in patients with end-stage renal disease on haemodialysis [102]

The SphygmoCor® Pulse Wave Velocity System provides a comprehensive assessment of arterial stiffness measured and useful assessment of the critical cardiovascular variables. It works by measuring the velocity of the blood pressure waveform between any two superficial artery sites, using a single-lead ECG and then tonometry to measure the pressure pulse waveform sequentially in the two sites. The tonometer is a delicate pressure transducer which is sited in a pen shaped probe, allowing ease of measurement when placed over the respective artery.

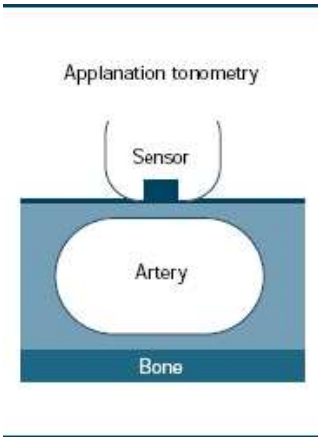

Figure 3.1 Principle of applanation tonometry; the artery is partially compressed against a hard structure.

Three blood pressure (BP) recordings are taken using an automated AND® UA-767 oscillometric device. A 3-Lead electrocardiograph (ECG) is attached to the subject and the surface distance between pulse points is measured using tape measure while the patient is supine. Thus calculations of velocity can be made (velocity= distance/ time). A single operator performs all haemodynamic measurements, reducing inter-observer error.

3.7.2 Aortic Augmentation Index

Peripheral wave reflection leads to augmentation of the aortic pressure wave. Augmentation index is a measure of the sum of the incident wave and the reflected wave, creating a systolic peak. This peak is thought to be a measure of the additional load to the left ventricle as a result of peripheral wave reflection. Augmentation index has been correlated with PWV as a marker for arterial stiffness as it is the vessel stiffness that affects the speed of the reflected wave.

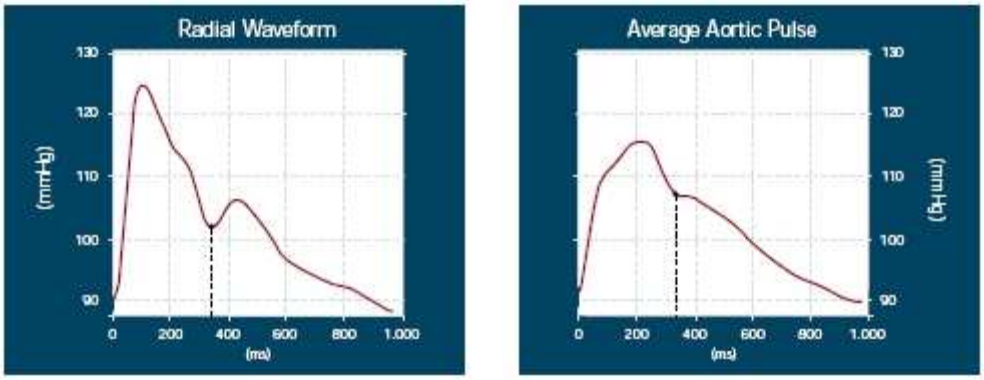

Figure 3.2 Standard arterial waveform for middle aged normotensive.

3.7.3 Reproducibility

In order to validate the results obtained using this technique, the inter-observer error between an experienced operator and investigator was assessed. Each operator made two PWA measurements on 10 consented subjects. Intraclass correlation coefficient analysis demonstrated a strong correlation between the two operators (0.86).

3.8 Local and distal microvascular assessment

3.8.1 Assessment of microcirculatory function

Microcirculatory function may be assessed indirectly (via surrogate humoral markers including asymmetric dimethylarginine and endothelins) or directly by assessing the vasodilator response to various stimuli, including ischaemia (typically post-occlusive), hyperthermia or drugs. Iontophoretic drug provocation with acetylcholine (ACh) and sodium nitroprusside (SNP) allows separation of the endothelial-dependent (ACh) and endothelial-independent (SNP) pathways. Both Laser Doppler flowmetery (LDF) and Laser Doppler imaging (LDI) utilize a low-power laser to detect blood flow in dermal capillaries, either at a fixed point (LDF) or scanning over a limited area (LDI). Their use has been demonstrated in CKD[103]. There are however, significant methodological constraints with both techniques, and thus specific care must be taken with many factors including the environment, site of assessment and drug delivery[104].

Cutaneous microcirculatory changes may have a direct bearing on the development and clinical course of vascular calcification (VC). The maintenance of flow to vulnerable critical visceral circulations is essential to maintain health, and abnormalities of microcirculatory function may be important in the overall pathophysiology of this process and the development of calcific uraemic arteriopathy. There are currently no data available on the distribution of directly calcified microvessels in patients with CKD.

Abnormal cutaneous microcirculation has been identified in patients receiving HD[105-106] and further reductions in microcirculatory function have been shown to be present in HD patients with large-vessel VC[107].

The cardiac microcirculation is becoming increasingly recognized as being important in the development of demand-induced myocardial ischaemia[108]. Impaired coronary flow reserve is determined by the maximum flow resulting from stress vasodilatation of both epicardial coronary arteries and the microcirculation. In health, 90% of MBF takes place through vessels <150 μm[109-110]. Myocardial ischaemia is well recognized as occurring in HD patients in the absence of large-vessel coronary disease[111].

There are no data currently available on the presence of significant calcification within the coronary microcirculation itself. Impaired microcirculatory function in ESRD patients is associated with increased LVM and arterial remodelling[112].

The assessment of subcutaneous dermal capillaries is a primary method for the assessment of the microcirculation. These vessels are representative of the microvascular supply to the heart[113] and kidneys[114]. This might allow study of the relatively accessible microcirculation to provide a window to critical central vascular beds. Abnormalities of endothelial function demonstrated using LDF can provide additional quantification of CV risk when used in conjunction with conventional risk scores[115]. LDF also appears to be able to document endothelial cell dysfunction in ESRF patients prior to clinical evidence of CV disease or diabetes mellitus[116].

3.8.2 Laser Doppler imaging (LDI)

Microcirculation is a collective term used to describe the smallest components of the cardiovascular system, namely arterioles, capillaries and venules. In order to identify the vascular changes related to microcirculatory flow and endothelial function we are using Laser Doppler Imaging (LDI) technique with iontophoresis of vasoactive drugs namely acetylcholine (Ach) and sodium nitroprusside (SNP). LDI is a fairly recent development [117] but results have been found to be closely correlated to capillary microscopy measurements of blood flow [118].

This technique will not only provide data regarding the local effects of the new fistula but comparison with data from the non fistula arm will also provide information on systemic and distal effects. Combining Iontophoresis of both Ach and SNP with this technique allows both endothelial dependant and non endothelial dependent vasodilatation responses to be measured.

Prior to starting the study, appropriate training was taken through a teaching course organised by the manufacturer in Stockholm – Sweden.

Studies are undertaken in a temperature controlled room following 10 minutes of rest. A laser beam scans over a designated area generating multiple measurement sites. By means of mirrors connected with stepper motors, the laser beam moves sequentially over the tissue over a measured site, over a set time [119]. Consecutive scans are then acquired. Dose-response information for vasodilator reactivity is obtained using transcutaneous

iontophoretic administration of the Ach and SNP. Two electrodes are applied to the surface of the forearm, filled with either 1% Ach or SNP and they are connected to a DC electric source for iontophoresis. A fixed current of 0.02 mA is used for 8 minutes for iontophoresing each drug. Cumulative dose-response curves are analysed automatically by the built-in software and data comparison can then be made between study days for individual subjects.

3.9 Haemodynamic assessment

3.9.1 Finometry

Systemic haemodynamic function is assessed non-invasively using a Finometer® (TNO Instruments Amsterdam, Netherlands). This technology utilises a finger-clamp method and allows detection in changes to digital arterial diameter by means of an infrared photoplethysmograph. This is opposed by an ultra-fast pressure servo controller that changes the pressure of an inflatable air bladder (also mounted in a finger cuff). Pulse wave analysis of the resultant arterial waveform and reconstruction of a central aortic waveform allows calculation a full range of haemodynamic variables on a continuous basis, for each heart beat [120]. These include pulse rate (HR), blood pressure (BP), stroke volume (SV), cardiac output (CO) and total peripheral resistance (TPR). All the data is subsequently downloaded to the

PC based analysis program Beatscope™ and results are averaged over a defined time period.

This process is conducted in a controlled environment after the participant has not eaten or consumed alcohol or coffee for at least 12 hours. Cuffs attached to the Finometer® are placed on an appropriate finger (ie middle finger, non-AVF side) and on the upper arm of the patient (non AVF).

3.9.2 Baroreflex Sensitivity (BRS) calculation

Autonomic dysfunction is common in patients receiving dialysis, and those with significant CKD. Short-term regulation of BP is largely controlled by appropriate autonomic nervous activity through the baroreflex arc[121]. Baroreflex sensitivity (BRS) is therefore well recognized as an integrated assessment of the autonomic nervous system[122]. BRS is not constant, and is affected by multiple factors. The involvement of drug treatment[123], endothelial dysfunction/paracrine factors[124], posture[125] and age[126] have all been demonstrated. Such dysfunction is associated with an increased incidence of cardiac arrhythmias[127], falls propensity, intradialytic haemodynamic instability[128], cardiac damage, metabolic syndrome[129], CV events and all-cause mortality after myocardial infarction[130] and in heart failure.

BRS as a marker of the fundamental control of BP is of great physiological significance in the study of HD. Impaired BRS has been demonstrated in

patients who are unstable on HD[131]. Furthermore, transfer from conventional haemodialysis three times weekly to nocturnal haemodialysis increases BRS[131], although further work is required to evaluate outcomes associated with what are at least theoretical benefits in terms of CV outcomes.

The finometer measures continuous interbeat interval (IBI) and beat-to-beat blood pressure changes. Baroreflex sensitivity (BRS) is a calculation of the regression slope between these two differentials. BRS software is integrated into the device and has been made available to use as a stand-alone DOS based offline analysis tool by Karl Wesseling (TNO instruments, Amsterdam). Three consecutive changes in the R-R interval in the same direction are required before a phase shift calculation (incorporated into the software) is performed and thus BRS is non-invasively measured with accuracy and precision. These variables vary with time so a recording of at least 10 minutes taken at rest is performed. BRS measured in this way is a composite marker of the overall activity of the autonomic nervous system [132].

3.10 Body composition and hydration status assessment

3.10.1 Bioimpedance

Measurements are made using the InBody S20 mutlichannel multisegmental analyser. This provides data on intracellular/extracellular fluid ratios, total body water (TBW), skeletal muscle and fat mass for the whole body, and segmentally for trunk and each limb. This technique is fully automatic

therefore interobserver variability is likely to be low. The manufacturers claim a measurement error of 1% with reproducibility of 99%.

It has been successfully validated against the 'gold-standard' of deuterium dilution for TBW assessment [133]. It has also been validated against DEXA for total and regional body composition in healthy subjects [134] and peritoneal dialysis patients [135].

3.11 Fistula flow assessment

3.11.1 Doppler ultrasound

Blood flowing through the fistula is determined by the sum of the flow resistance of the arterial system between the left ventricle and the anastamosis, the flow resistance of the anastamosis itself and the flow resistance of the venous system between the anastamosis and the right atrium [64]. Colour flow Doppler provides accurate imaging and flow volume measurement of vascular access in haemodialysis. Doppler ultrasound has become the standard of care for evaluation of AVF dysfunction and is essential in the preoperative evaluation for access placement [136].

As well as pre-operative vascular mapping, post operative evaluation of the fistula flow is carried out using an ultrasound colour-flow scanner. Fistulae are scanned with a linear array transducer, longitudinally and transversely All vascular flow measurements were done by a single experienced user using a dedicated vascular probe (4-10 MHz 10L-Linear Probe, GE medical systems).

Vascular ultrasound/Doppler training was by delivered by highly experienced senior sonographers for the period of 6 months. Training has been undertaken with regards to vascular mapping prior to AVF creation, measuring blood vessel diameter and timed average velocity of blood flow and calculating flow rate. Cross sectional area measurements are made by tracing the vessel outline under maximum resolution and the accuracy and quality of the images taken were assessed. Changes in velocity, volume, fistula size and are recorded at each assessment stage and results compared.

3.12 Laboratory Tests

Routine blood tests performed as part of standard patient care will be recorded. No additional blood tests will be requested as part of the study at this time. However, it is possible that testing for BNP, CRP or other biochemistry may become relevant as this study progresses. Thus, we are saving serum from participants during the study to allow analysis later, if required. We will not use these samples for any genetic testing or to produce results that would have a direct prognostic effect on a particular patient. If these samples are not used they will be discarded at the end of the study.

3.13 Statistical considerations

Continuous consultation with the University of Nottingham research statistician occurs in relation to this study. Assistance was initially required in regards to power calculations and analysis planning.

3.13.1 Primary outcome measure

'To detect a significant difference in arterial stiffness, as measured by pulse wave velocity.'

A change of one metre per second was to be identified, comparing patients pre AVF to the repeat assessments performed at two weeks and nine months post AVF surgery. Using data generated by previous published research from Derby we are able to perform the following power calculations for this study. Using work by Sigrist et al [137] we can use the mean value and standard deviation obtained for carotid-radial PWV of patients with pre-dialysis CKD.

Mean PWV = 9m/s

Standard deviation = 1.6 m/s

Observing a significant difference of 1m/s, at 6/52 and 6/12.

Power = 90%

Significance level of = 5%

The required sample size was 29 patients. Adjusting for a failure rate of 30% for primary AVF (local rates) and 15% annual mortality the final sample size needed for this study was 50 patients.

3.13.2 Secondary outcomes measures

1. Cardiovascular function

Changes in LV systolic function (global ejection fraction). Pre and post AVF formation echo data was compared.

2. Microvascular function

Dose response curves generated by laser Doppler allowed comparison of microcirculatory flow, comparing results pre and post AVF as well as comparison of the AVF arm to the non AVF arm.

3. Fistula flow

Vessel diameter, cross sectional area, timed average velocity and volume are measured by the Doppler ultrasound. Changes over time were analysed.

4. Haemodynamic function

Average recording over 10 minutes provided data on heart rate (HR), blood pressure (BP), stroke volume (SV), cardiac output (CO) and total peripheral resistance, allowing data analysis.

5. Autonomic function (BRS)

Calculation of average change in interbeat interval for a change in blood pressure of 1mmHg compared to ms/mmHg. Changes in BRS associated with AVF formation and maturation will be assessed.

Chapter 4

Effects of AVF creation on systemic haemodynamics and left ventricular systolic function

4 Results: Effects of AVF creation on systemic haemodynamics and left ventricular systolic function

4.1 Introduction

Upper extremity native arteriovenous fistula (AVF) is the vascular access of choice, supported by the KDOQI of the National Kidney Foundation[138]. The use of definitive vascular access in HD patients, rather than tunnelled central venous catheters, is associated with sustained reduction in mortality over at least three years[75]. This difference in survival has previously been entirely attributed to differences in access related sepsis. Little is currently known concerning the systemic structural and functional cardiovascular changes that occur as a consequence of AVF formation. Several case reports indicate that very high fistula flows (Qa) can be associated with inducing a high cardiac output status and subsequent precipitation of cardiac failure[88-89 139], and the presence of an AVF may modulate sympathetic outflow[88] and markers of volume status such as BNP[140].

However, there have been no prospective studies of the totality of effect of de novo AVF formation on cardiovascular system structure and function.

It is well recognised that dialysis patients display hugely elevated rates of cardiovascular mortality[141]. It is also becoming appreciated that this rate of cardiovascular attrition is not driven by the same variety of risk factors, or pathophysiological processes that are important in the general population[142].

Cardiac failure develops in as many as 25-50% of HD patients and confers a dramatic reduction in probability of survival[143]. HD patients are particularly susceptible to demand myocardial ischaemia. In addition to the high prevalence of coronary atheroma[144] , diabetic dialysis patients have been shown to have a reduced coronary flow reserve, even in the absence of coronary vessel stenoses[145].

The aim of this study is to prospectively investigate the acute effects of AVF formation on systemic haemodynamics and left ventricular function as these factors are crucial in the pathophysiology of CV diseases in this group of patients.

4.2 Methods

4.2.1 Subjects

Recruitment of the initial 43 patients is covered in chapter 3, section 3.2 and it is not repeated here.

Successful AVF formation was defined as an arteriovenous anastomosis with clinical (palpation and auscultation) and Doppler ultrasound confirmed blood flow two weeks postoperatively.

All the subjects were studied two weeks prior to their planned operation date and restudied 2 weeks postoperatively. No acute illness or major post operative complication was recorded during the follow up period. In addition, all the vasoactive medications (B-blockers, calcium channel blockers, angiotensine converting enzyme inhibitors, aldosterone receptor blockers, diuretics and alpha blockers) remained unchanged during the study period.

4.2.2 Haemodynamic studies

On the study days, patients were allowed to rest in bed for 15 minutes before data collection. Three blood pressure recordings were taken using an automated AND® UA-767 oscillometric device on the non-fistula arm. Haemodynamic measurements were taken non-invasively using Finometer® (Finapres Medical Systems, Arnhem, the Netherlands). This technology uses pulse-wave analysis obtained at the digital artery to measure beat to beat blood pressure and heart rate. Modelflow™ derived changes in cardiac output

(CO), stroke volume (SV) and total peripheral resistance (TPR) were calculated using a reconstructed aortic pulse wave. BRS values were recorded using software analysing the relationship between inter-beat interval variability and beat to beat changes in systolic BP[146].

4.2.3 Echocardiographic and Doppler ultrasound studies

All studies were conducted during first and second visits using Vivid 3® ultrasound machine with a dedicated cardiac probe ((1.5-3.6 MHz 3S probe, GE medical systems, Sonigen, Germany). M-mode ventricular parameters were measured according to the recommendations of the American Society of Echocardiography[101]. A single experienced operator carried out all measurements with the patients in the left lateral position. Echocardiographic evaluations were performed by another experienced observer (TE) who was completely blinded to operation outcome, examining the digitized images obtained for off line analysis. Ejection fraction (EF) was calculated using LV volume at end systole and end diastole obtained from M-Mode echocardiography images.

All vascular flow measurements were done by a single experienced user using a dedicated vascular probe (4-10 MHz 10L-Linear Probe, GE medical systems). All patients were studied in sitting position. Examination followed the blood flow from the afferent artery into the anastomosis, the access vein and the draining veins. All the flow rate measurements were done in a straight access segment free of turbulent flow. The transverse diameter of the vessel

was multiplied by the average flow velocity to obtain the volume flow rate in ml/min.

4.2.4 Body Composition and blood tests

Bioelectrical impedance was measured using InBody S20® body composition analyser (Biospace, Korea) to detect changes in total body water and soft tissue composition. Blood samples were collected during each study session and biochemical analysis including full blood count, electrolytes, urea, creatinine, bone profile, albumin, C-reactive protein and Troponin T serum levels were measured.

4.3 Statistical analysis

Normality of distribution of the data was tested using Shapiro-Wilk tests test. Results are expressed as mean ± SD. For comparing pre- and post operative data, paired *t*-test was used. An alpha error at $P<0.05$ was judged to be significant.

Simple linear relationship between different variables was measured using Pearson's coefficient of correlation.

Stepwise multivariate regression analysis was performed to determine independent predictors of fistula blood flow (Qa). Factors for the models were considered either because they were established determinants of the dependant variable (Qa) or they achieved significant value in the initial

regression analysis using “enter” method. All statistical analysis was performed using the SPSS software package, version 12 (SPSS Inc., USA).

4.4 Results

4.4.1 Baseline characteristics

All patients had an end-to-side anastomosis and the operations were done as a day case procedures under local anaesthetic. One patient died before attending the second study session, from unrelated causes. Two patients who had an unsuccessful AVF operation initially, were re-recruited and consequently had a successful AVF formed.

30/43 patients had successful AVF operation performed (22/29 brachiocephalic, 3/3 brachiobasilic and 5/11 radiocephalic). Patients with failed AVF (13/43) procedures were utilised as sham operated controls. Demographic, biochemical, and CV risk factors characteristics of the study participants according to their operation outcome are listed in table 4.1.

Mean age was 68±13 years. Of those studied, 40% were female and 60% male. The causes for chronic renal failure amongst the cohort were diabetic nephropathy 37%, primary glomerulonephritis 14%, unknown 21% and other causes (polycystic disease, vasculitis, renovascular diseases and obstructive uropathy) 28%. None of the patients in the cohort had a documented clinical diagnosis of cardiac failure. However, our analysis of the echocardiographic images obtained during baseline sessions showed that EF was less than 50% in 15/30 patients with successful AVF.

	Unsuccessful AVF (n=13)	Successful AVF (n=30)	*P*
Age	66.7±15	68.7±12	0.7
Gender(male:female)	6:7	20:10	0.2
BMI	30.5±5.2	28.6±6.3	0.3
Ischemic heart disease	39%	37%	0.9
Diabetes	46%	37%	0.5
Hypertension	92%	77%	0.1
Dyslipidaemia	69%	70%	0.9
Smoking	69%	60%	0.6
ACE inhibitors/ARB	62%	73%	0.4
Pre eGFR (ml/min)	17±4	17±4	0.2
Albumin (g/l)	37±3	36±4	0.7
Hb (g/dl)	11.8±1.3	11.4±1.4	0.3

Table 4.1 Demographic, biochemical and cardiovascular risk factors in both groups

BMI=Body Mass Index, ACE inhibitors=Angiotesin-Converting Enzyme inhibitors ARB=Angiotensin Receptor Blockers, eGFR= estimated Glomerular Filtration Rate

4.4.2 Haemodynamic data

The baseline and postoperative haemodynamic data in both patient groups are summarized in table 4.2. These significant haemodynamic changes only occurred in patients who had a successful AVF formation. Two weeks postoperatively, peripheral systolic and diastolic BPs were both decreased (-9.7±18 and -9.5±10.3 mmHg respectively). This was accompanied by a similar reduction in central systolic BP -12.4±16.2 mmHg and central diastolic BP -7.8±7.9 mmHg. Total peripheral resistance decreased (-0.2±0.021 mmHg.sec/ml, p=0.001) stroke volume tended to increase (12±30 ml, p=0.053) and heart rate increased (4±8.0 bpm, p=0.01). This was associated with an increase in cardiac output (1.1±1.5 L/min, p=0.001).

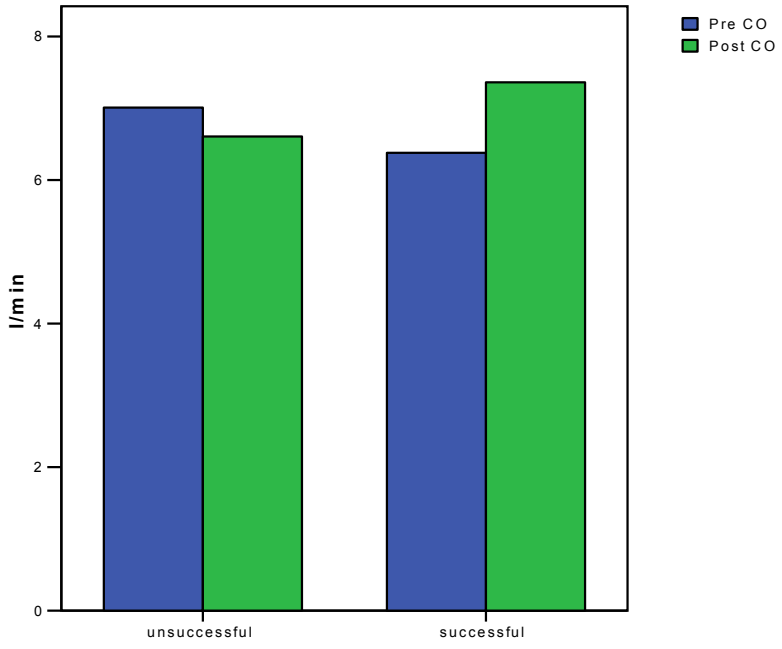

Figure 4.1 Mean cardiac output (CO) in both groups pre and 2 weeks postoperatively

Parameter	Successful AVF				Unsuccessful AVF			
	Pre	Post	%	p	Pre	Post	%	p
Peripheral SBP	144±28	134±21	-5±13	0.006	137±19	138±20	1	0.6
Peripheral DBP	75±12	66±11	-12±13	0.001	74±12	75±13	-1	0.7
SV(ml)	113±33	124±41	11±22	0.053	116±41	116±41	1	0.9
HR(bpm)	60±11	64±10	8±14	0.01	61±11	59±7	-3	0.3
CO(l)	6.5±1.5	7.6±2	19±25	0.001	7±3	6.8±2.5	-3	0.7
CI(l/min/m^2)	3.45±0.7	4.1±1.0	19±25	0.001	3.7±1.6	3.5±1.2	-3	0.7
TPR(mmHg.sce/ml)	1.0±0.2	0.8± 0.2	-17±18	0.001	1.0±0.4	1.1±0.7	15	0.4
BRS	5.6±3	5.8±2.5	10±38	0.4	5.8±3.4	6±3.1	25	0.2
EF(%)	45±13	52±12	6±8	0.001	45±13	45±14	-1	0.8

Table 4.2 Baseline and postoperative haemodynamic data in both groups

4.4.3 Body composition and laboratory data

There were no significant changes in total body water (intra and extra cellular water) and soft tissue composition in either group postoperatively. Furthermore, no significant differences in any of the biochemical and haematological parameters were observed in either group postoperatively. There was no change in renal function as measured by estimated GFR.

4.4.4 Echocardiographic and Doppler ultrasound

Two weeks postoperatively, patients who had a successful AVF formation demonstrated an increase in their ejection fraction (6.5±8.5%, p=0.001). This was in contrast to those patients with unsuccessful AVF who showed no change (figure 4.2). Not all patients exhibited an increase in EF, 5/30 had a small mean reduction (-2.6±1.1%). These patients were older (75±9) with higher baseline systolic BP and had stiffer arteries (see table 4.3). However, their arterial stiffness indices, BPs and TPR were reduced after AVF formation in similar way to the others.

Clinically, no case of cardiac decompensation was recorded following AVF creation.

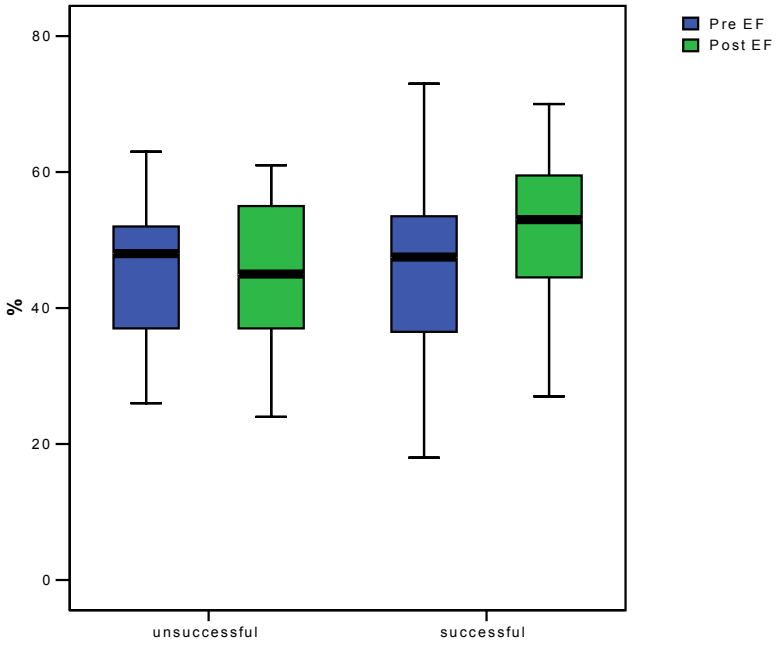

Figure 4.2 Left ventricular ejection fraction (EF) in both groups

pre and postoperatively

Parameter	Increased EF		Reduced EF	
	Pre	Post	Pre	Post
Peripheral SBP	142±28	132±20	155±25	138±20
Peripheral DBP	76±13	64±12	74±3	69±6
Central SBP	132±27	118±19	143±23	134±19
Central DBP	73±14	65±12	75±3	70±6
CF-PWV(m/s)	12.2±3	10.9±3	13.4±2	12.1±2.4
AIx%	22±10	18±10	25±8	22±8
SV(ml)	117±27	127±23	84±27	86±12
HR(bpm)	60±13	64±10	60±10	62±10
CO(l/min)	6.6±1.5	7.2±1.4	5.2±1	6.2±0.5
CI(l/min/m^2)	3.5±0.7	3.8 ±0.66	3.25±0.83	4.2±1.58
TPR(mmHg.sec/ml)	0.97±0.17	0.83± 0.17	1.18±0.4	0.93±0.3

Table 4.3. haemodynamic and arterial stiffness parameters pre and postoperatively in the patients with increased EF compared to patients with reduced EF (n=5) two weeks postoperatively.

Mean AVF flow (Qa) varied according to the type of the fistula constructed; with brachiobasilic fistulas exhibiting mean Qa of 1500±1100 ml/min, brachiocephalic fistulas 1300±600 ml/min and radiocephalic fistulas 600±170 ml/min. Diameter of the artery and vein designated for the AVF formation and blood flow through the artery preoperatively were statistically different between both groups. These data are summarised in Table 4.3.

	Unsuccessful AVF (n=13)	Successful AVF (n=30)	*P*
Artery diameter (mm)	33±10	45±12	0.007
Artery blood flow (ml/min)	43±22	77±44	0.01
Vein diameter (mm)	32±10	40±11	0.07

Table 4.4 Preoperative blood vessels diameter and arterial blood flow in both groups

On a multivariate regression analysis, the major determinant of the Qa flow were diameter of the AVF itself and the blood flow in the supplying artery (R^2=0.63, p=0.001 and 0.02 respectively, (see table 4.4). There was no effect of cardiac performance on AVF blood flow.

Variables	Unstandardized Coefficients		Standardized Coefficients	P
	B	Std. Error	Beta	
AVF diameter	212.8	55.526	0.548	0.001
Post operative arterial flow	0.351	0.145	0.347	0.022

Table 4.5 Multivariate analysis of factors affecting Qa

4.5 Discussion

Although there has been some study of the effect of AVF formation on isolated aspects of the CVS, this is the first study to prospectively investigate the observed effects in detail on cardiovascular structure and function (both peripheral and cardiac). Furthermore, it is the first study to exclusively investigate the effects in patients without previous vascular access or exposure to dialysis. The utilisation of patients with primary technical failure provides the opportunity to study those effects controlled against a group of patients undergoing the same progression of uraemic factors, but without a formed shunt.

From the physiological studies looking into compensation of CO and other haemodynamic variables in animal models post AVF formation, it is believed that increase in CO is mediated by two mechanisms[88]. The acute response which happens within seconds of AVF formation is caused by increased in venous return secondary to reduction in TPR and cardiac adaptation, in accordance with Starling's principle. Subsequent responses appear to occur secondary to neurohormonal and autonomic nervous system modifications.

The significant increase in the CO demonstrated in our study, is similar to the observation made by the few other clinical studies which have looked into the effect of AVF formation on cardiac output[89 140 147] (utilising different techniques to measure central haemodynamics).

The prevailing view that higher flow within the AVF is associated with high output cardiac failure is widely held. However this assertion has not been

previously tested in prospective rigorous study. Uniquely, we have investigated the changes in systemic haemodynamics including CO specifically in pre-dialysis population, without many of the confounders that would be involved in using a dialysis based population (changing volume status, resolution of uraemia, medication changes etc.). All patients who had a successful AVF formed showed an increase in their CO, however there did not appear to be any relationship between higher Qa values and CO. There was no clinical evidence of cardiac decompensation, with no change in body water. Echocardiography data demonstrated that even two weeks postoperatively, there was a significant increase in EF. This is similar to changes noted in previous studies[140 147]. The observed change in EF continued to be persistent up to three months postoperatively when the vascular access had matured (see chapter 6).

Preoperatively, 50% of the patients who had successful AVF formed had EF values of less than 50%. In contrast to the common belief of avoiding AVF placement in patients with impaired ventricular function, these patients underwent proper assessment and had their AVF created. Echocardiography data demonstrated that even two weeks postoperatively, there was a significant increase in EF. This is similar to changes noted in previous studies[140 147]. Interestingly, there was no clinical evidence of cardiac decompensation, with no change in total or ECW water in any of the patients studied. The above finding suggests that the practice of withholding AVF placement in older patients or those who have CV compromises may well

need to be revised. This point is more comprehensively discussed in the following chapters of this thesis.

4.5.2 Conclusion

Formation of an AVF resulted in significant changes in systemic haemodynamics. Overall the post AVF adaptations might be characterised as potentially cardioprotective.

Left ventricular ejection fraction increased and there was no evidence of acute cardiac decompensation in this acute setting, and further study of the longer term CV responses to AVF formation are essential to improve our understanding of the complex role that vascular access choices might make on patient outcomes.

Chapter 5

Effects of AVF creation on arterial stiffness, central and peripheral blood pressures

5 Results: Effects of AVF creation on arterial stiffness, central and peripheral blood pressures

5.1 Introduction

CKD patients are known to have a blood pressure values characterised by elevation of systolic blood pressure and normal or even reduction in their diastolic blood pressure[148]. These readings are associated with increased stiffness of the large conduit arteries. The exact cause behind increase in arterial stiffness in CKD patients is not completely understood but it is not exclusively related to increase in blood pressure, increased wall stress or other conventional CV risk factors such as blood glucose, body weight, cholesterol or smoking[149-150]. Increased arterial stiffness leads to alteration of central blood pressures, increasing myocardial oxygen demand whilst reducing supply due to the reduction in diastolic blood pressure reinforcement from the returning pressure wave. These factors increase the risk of myocardial hypoperfusion[151].

Studies of myocardial blood flow during HD in adult patients with normal coronary angiograms[152] and dialysis induced cardiac segmental ischaemia in children on haemodialysis[153], have highlighted the existence of demand myocardial ischaemia during HD in the absence of atheromatous large vessel coronary artery disease. Such repeated injury results in systolic dysfunction, increased cardiac arrhythmias and markedly reduced survival[154-155] .

It is already been shown that increased arterial stiffness is associated with impairment in creatinine clearance in patients with mild to moderate CKD independent of age, blood pressure and other risk factors[156]. It has also been demonstrated that arterial stiffness increases across a range of patients with different stages of CKD as eGFR increases[58].

Furthermore, in patients with ESRD, for 1m/s increased arterial stiffness, all-cause mortality-adjusted OR was 1.39 (95% CI, 1.19 to 1.62)[60]. When hypertensive, ESRD patients treated with antihypertensives, it was the ΔPWV which was a direct predictor of all cause mortality independent of reduction in blood pressure[157].

The impact of AVF creation on arterial stiffness (as a critical determinant of potential demand ischaemia and increased mortality) has not been studied before. The few studies that have been performed have tended to be small, focus on only limited elements of the cardiovascular system and/or rely on extrapolation of data derived from closure of troublesome AVFs in established HD patients.

We already know that fistula creation is associated with significant haemodynamic changes including reduction in BP and TPR and increase in SV, HR and CO. The aim of this study is to (1) detect any significant changes in arterial stiffness in response to AVF creation and (2) look into the association between change in arterial stiffness and other haemodynamic variables.

5.2 Methods

5.2.1 Patients

Recruitment of the initial 43 patients is covered in chapter 3, section 3.2 and it is not repeated here.

Successful AVF formation was defined as an arteriovenous anastomosis with clinical (palpation and auscultation) and Doppler ultrasound confirmed blood flow two weeks postoperatively.

All the subjects were studied two weeks prior to their planned operation date and restudied 2 weeks postoperatively. No acute illness or major post operative complication was recorded during the follow up period. In addition, all medications remained unchanged during the study period.

5.2.2 Study protocol

All patients gave consent prior to commencement. Patients were studies at the same time of the day after abstaining from smoking, caffeine and smoking for at least 12 hours. On the study days, patients were allowed to rest in bed for 15 minutes before data collection. Three blood pressure recordings were taken using an automated AND® UA-767 oscillometric device on the non-fistula arm.

As a measure of arterial stiffness, the gold standard carotid-femoral pulse wave velocity (CF-PWV) was taken using Sphygmacor® (AtCorTM, PWV Inc., Westmead, Sydney, Australia) pulse wave velocity system.

In addition, Aortic Augmentation Index (AIx) and central blood pressure were measured from radial artery tonometry. All CF-PWV and AIx measurement were repeated three times and the average calculated for subsequent analysis.

This protocol was repeated for the subsequent study sessions.

5.3 Statistical analysis

Sample size was calculated with reference to the primary outcome of change in PWV with formation of an AVF. Details about the study power and the statistical tests used in the analysis are discussed in chapter 3, section 3.13.

Stepwise multivariate regression analysis was performed to determine independent predictors of change in CF-PWV (Δ PWV). Factors for the models were considered either because they were established determinants of the dependant variable (ΔPWV) or they achieved significant value in the initial regression analysis using “enter” method. All statistical analysis was performed using the SPSS software package, version 12 (SPSS Inc., USA).

5.4 Results

5.4.1 Baseline characteristics

All patients had an end-to-side anastomosis and the operations were done as a day case procedures under local anaesthetic. One patient died before attending the second study session, from unrelated causes. Two patients who

had an unsuccessful AVF operation initially, were re-recruited and consequently had a successful AVF formed.

30/43 patients had successful AVF operation performed (22/29 brachiocephalic, 3/3 brachiobasilic and 5/11 radiocephalic). Patients with failed AVF (13/43) procedures were utilised as sham operated controls. For demographics, biochemical and cardiovascular risk factors of the study participants according to their operation outcome please see chapter 4, section 4.4.1 and table 4.1.

5.4.2 Blood pressure

Table 5.1 shows the effect of AVF creation on central and peripheral BPs, CF-PWV and AIx in both patients group pre and postoperatively. Significant changes only occurred in patients who had a successful AVF formation.

Two weeks postoperatively, peripheral systolic BP was significantly reduced (-9.7±18 mmHg) as was diastolic BP (-9.5±10.3 mmHg), see figure 5.1 and 5.2.

Parameter	Successful AVF				Unsuccessful AVF			
	Pre	Post	%	P	Pre	Post	%	p
Peripheral SBP	144±28	134±21	-5±13	0.006	137±19	138±20	1	0.6
Peripheral DBP	75±12	66±11	-12±13	0.001	74±12	75±13	-1	0.7
Central SBP	133±26	121±19	-8±12	0.001	127±17	129±19	1	0.6
Central DBP	73±13	67±11	-9±11	0.001	76±12	75±13	-2	0.8

Table 5.1 Baseline and postoperative blood pressure data in both groups

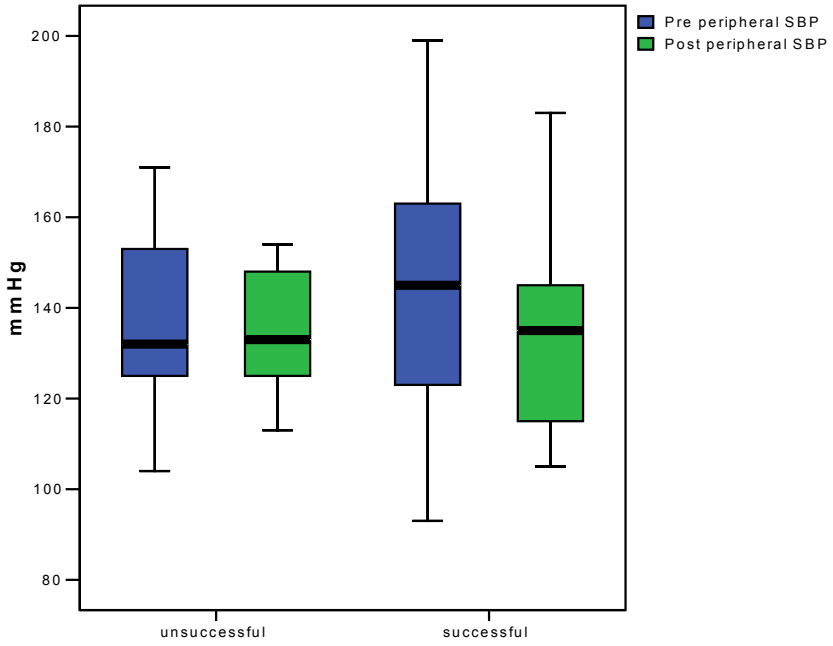

Figure 5.1 Peripheral systolic blood pressure (SBP) pre and postoperatively in both groups

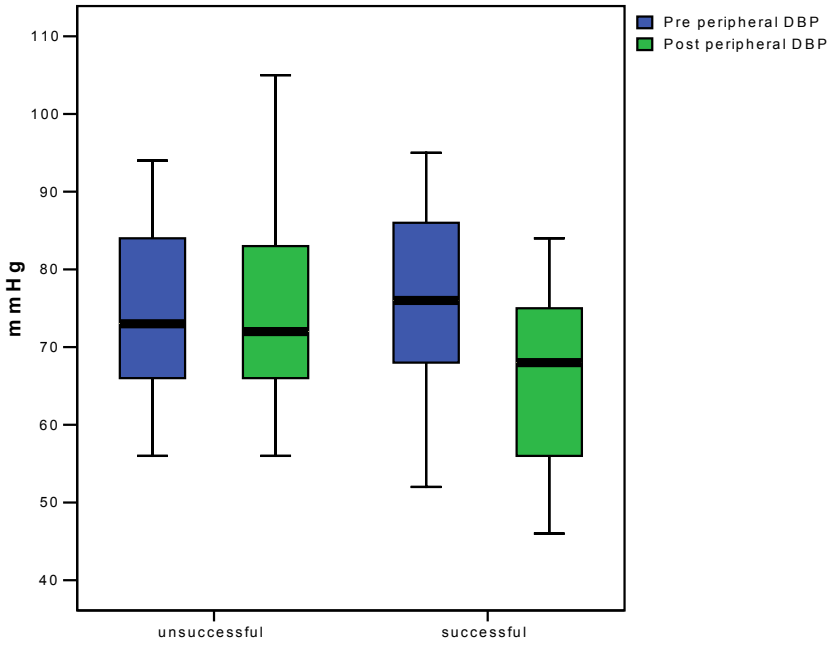

Figure 5.2 Peripheral diastolic blood pressure (DBP) pre and postoperatively in both groups

This was accompanied by a similar reduction in central systolic BP (-12.4±16.2 mmHg) and central diastolic BP (-7.8±7.9 mmHg), see figure 5.3 and 5.4.

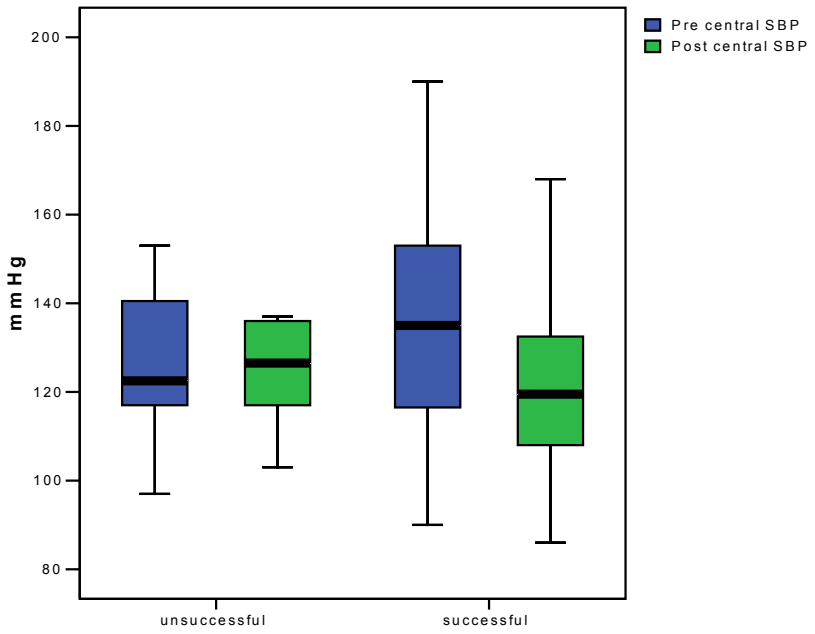

Figure 5.3 Central systolic blood pressure (SBP) pre and postoperatively in both groups

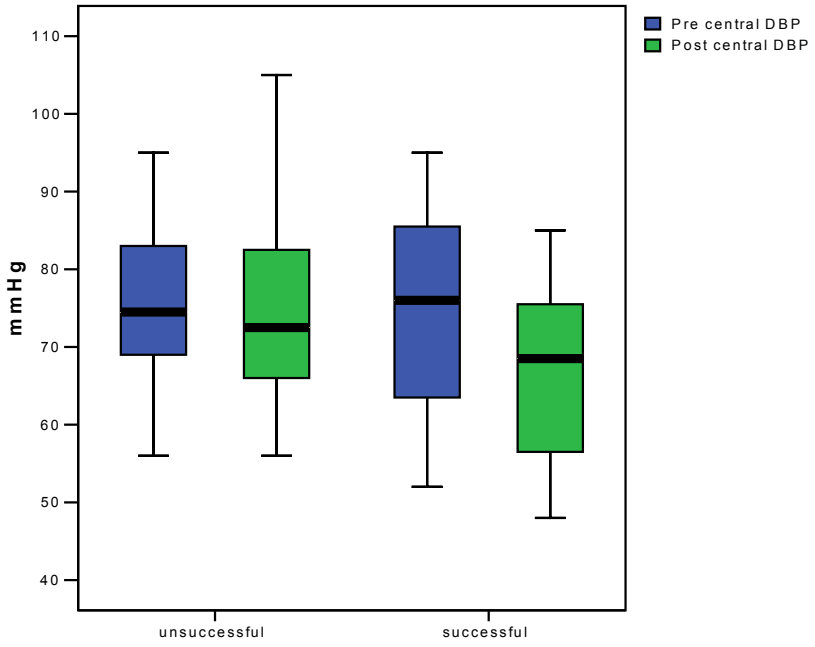

Figure 5.4 Central diastolic blood pressure (DBP) pre and postoperatively in both groups

5.4.3 Arterial stiffness

Arterial stiffness markedly decreased following successful AVF formation. CF-PWV was reduced by a mean of (-1.1±1.5 m/sec, p=0.004, figures 5.5). AIx was also reduced (-3.7±5.4%, p=0.002) significantly in patients who had a successful AVF formed (see table 5.2 and figure 5.6).

In a stepwise multivariate analysis, change in peripheral diastolic BP (∆D-BP) and the presence of diabetes mellitus were independently associated with observed ∆PWV (R^2=0.393, p=0.001), with ∆D-BP independently contributing by 25% to the model (R^2=0.25, p=0.004).

.Parameter	Successful AVF				Unsuccessful AVF			
	Pre	Post	%	P	Pre	Post	%	p
CF-PWV(m/s)	12.6±3.5	11.0±3	-8±13	0.004	10.8±2	10.9±2.4	1	0.7
AIx%	22±9	19±9	-15±30	0.002	25±9	23±9	-2	0.4

Table 5.2 Baseline and postoperative measurements of arterial stiffness indices in both groups

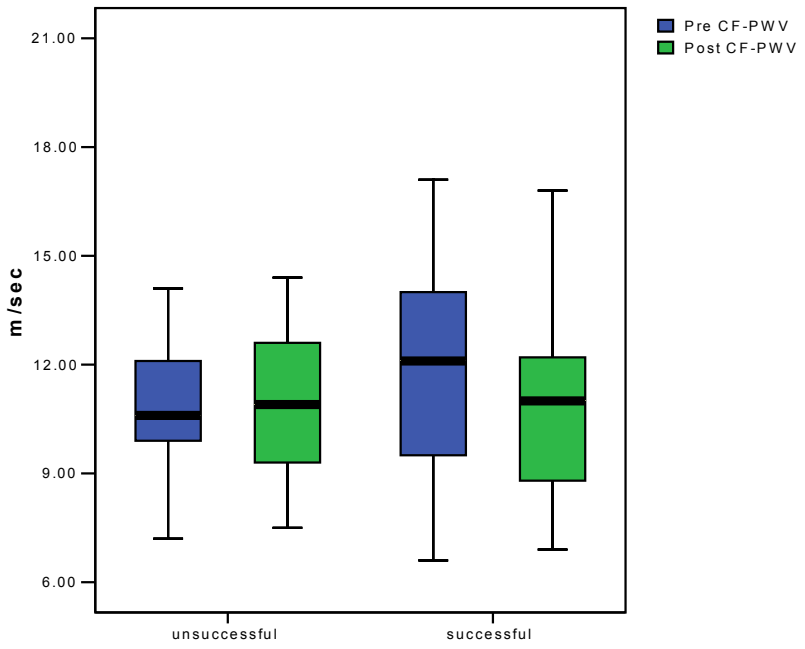

Figure 5.5 Carotid Femoral Pulse Wave Velocity (CF-PWV) pre and postoperatively in both groups

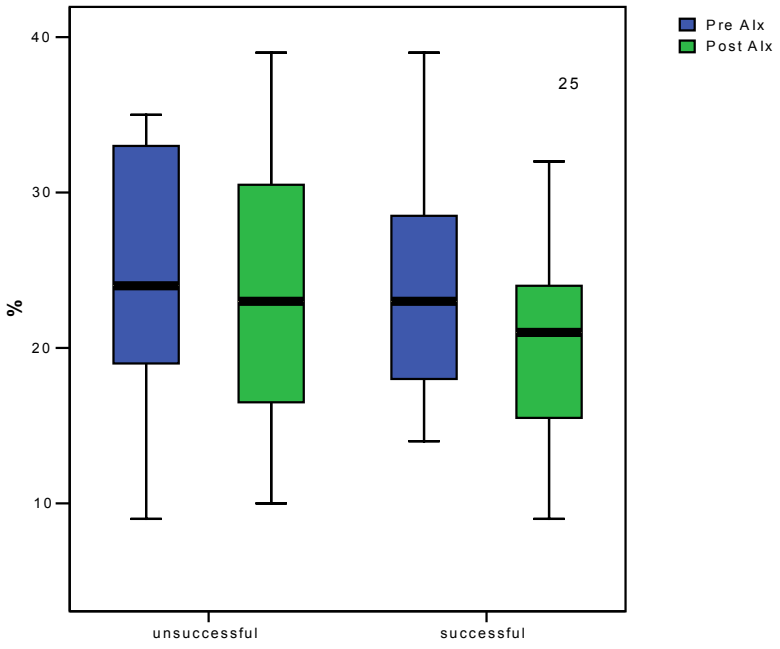

Figure 5.6 Aortic Augmentation Index (AIx) pre and postoperatively in both groups

5.5 Discussion

This study prospectively investigates the effects of AVF formation on peripheral and central haemodynamics and cardiovascular structure and function. It exclusively investigates the effects in patients without previous vascular access or exposure to dialysis.

We have demonstrated that both central and peripheral blood pressure fell significantly after AVF formation. This can be attributed to the described fall in TPR (see chapter 4). Interestingly even this fairly simple consequence of AVF formation has been subjected to very little formal study. Ori *et al* also showed reduction in systolic and diastolic blood pressure, but to a lesser extent, possibly due to a smaller sample size (n=10)[147].

We also looked into the association between AVF formation and CF-PWV allowing correlation with changes in other haemodynamic variables. Savage *et al* measured AIx in nine patients, reporting this composite marker of arterial compliance and cardiac performance did not change significantly after AVF formation[158]. More recently, Utescu *et al* demonstrated that successful AVF formation was associated with reduction in CF-PWV and mean BP and a non significant increase in AIx[159]. Interestingly, although baseline CF-PWV and the decrease in CF-PWV over 3 months are similar in our study to the study by Utescu *et al*, our present study showed that as markers of arterial stiffness, both CF-PWV and AIx were significantly reduced 2 weeks postoperatively and it persisted for 3 months afterwards (see chapter 6). One of the crucial differences between the two studies is that none of our patients were

commenced on haemodialysis treatment before the last study session. In contrast Utescu *et al* reported that 52% of their participants were already receiving HD at the beginning of the study and this increased to 71% at the end of the study. Haemodialysis per se has been reported to affect both AIx and PWV measurements significantly[160-162]. Recognizing this fact, we deliberately designed our study to avoid potential effects of haemodialysis on arterial stiffness, haemodynamics and body composition parameters. It is also widely recognized that although PWV and AIx are two different measurements of arterial stiffness, they are not interchangeable or superimposable[160]. These important factors could well explain the difference in AIx trend between the two studies.

Although assuming that AVF creation affecting the structure of the arterial wall in such as short period is less likely and out there to be proven, it seems that reduction in arterial stiffness parameters is largely associated with reduction in the BPs. Indeed when we closely examined the relationship between the change in CF-PWV and other haemodynamic variables, to determine if reduction in CF-PWV can be partially explained by the changes in the other haemodynamic variables, it was determined that changes in peripheral diastolic BP remained the most significant determinant of change in CF-PWV contributing around 25% to the model. None of the other haemodynamic variables including changes in SV, TPR and HR contributed significantly. This finding was also very similar to findings by Utescu *et al*.

As arterial stiffness has been previously demonstrated to be associated with cardiovascular risk factors and cardiovascular diseases in pre dialysis population [163], we hypothesize that this reduction in CF-PWV may well contribute to improved outcome in patients who have native AVF compared to other types of dialysis access.

5.5.3 Conclusions

This study shows that AVF creation is associated with significant reduction in arterial stiffness and blood pressures two weeks postoperatively. The reduction in CF-PWV is associated but not completely explained by reduction in blood pressure.

These described changes could be beneficial in longer term and could well contribute; at least partially; to lower all cause and cardiovascular mortality in CKD patients with AVF as vascular access compared to those with other types of access for dialysis.

Chapter 6

Longer term haemodynamic consequences of AVF creation

6 Results: Longer term haemodynamic consequences of AVF creation

6.1 Introduction

The data contained in the previous chapters demonstrates that AVF creation is associated with acute significant changes in systemic haemodynamics, myocardial contractile function, arterial stiffness and microcirculation reserve.

The longer term consequences of AVF formation have not been studied in great detail previously and unfortunately, little is known about if effects of AVF creation contribute to better survival in dialysis CKD population. What is known though is that CKD patients who dialyse through a native AV fistula have reduced all cause mortality compared to others dialysing through AV graft and, even worse through tunnel venous catheters[164]; however; beside reducing infection rate, it is still unknown if there are other factors contributing to improved survival in patients with AVFs.

Reasons for paucity of studies looking into longer term effects of AVF creation could be the fact that patients who had AVF created went onto dialysis sooner which is associated with significant haemodynamics and body composition alteration itself and secondly, rate of fistula failure and complications increases as it is utilised for dialysis, rendering longer term follow up studies rather complicated and difficult to design.

This part of the study was designed to investigate the longer term (up to 3 months) consequences related to AVF creation. It was designed as an

integral part of the original research study aiming to investigate both acute and longer term effect of AVF creation prior to patients starting dialysis.

6.2 Methods

6.2.1 Patients

Recruitment of patients are covered in chapter 4, section 4.2.1. Of the initial 30 patients who had successful AVF created, 21 patients consented for the 3 months follow up study. Patients who were censored included those who had died (n=2), patients who started haemodialysis (n=3) and patients who declined to participate in further investigations (n=4). Patients who did not have successful AVFs were not entered into the 3 months follow up session.

6.2.2 Study protocol

3 months postoperatively, patients were approached to verbally confirm their consent to stay in the study.

As near to 3 months as practical, patients underwent an identical study session to the ones at the baseline and 2 weeks postoperatively. Their medical history, progression of CKD, start date for dialysis and drug history was reviewed and compared with the medical notes.

During 3 months follow up session, the following variables were collected:

- Haemodynamics including CO, HR, TPR and SV.
- Arterial stiffness: CF-PWV and AIx.

- Echocardiography: M-mode and two dimensional two and four chamber views
- Doppler ultrasound: to measure vessels diameter and AVF flow.
- Bioimpedence analysis: measuring body composition and hydration status.

Primary end point was to assess the change in arterial stiffness measured by CF-PWV. We already have demonstrated that CF-PWV is reduced significantly 2 weeks after AVF creation. The aim was to determine if this reduction was sustainable or reversible. In addition, we sought to identify the consequences of AVF creation on left ventricular ejection fraction, blood pressures and cardiac output after 3 months as secondary end points.

6.2.3 Follow up collection of study data

Haemodynamic, echocardiographic, Doppler, arterial stiffness, body composition and blood tests data were collected similar to the previous study sessions as outlined in chapter 4, section 4.2.2 to 4.2.4 and is not repeated here.

6.3 Statistical analysis

Power of the study and the tests used in statistical analysis are all described in chapter 4, section 4.3 and chapter 5, section 5.3. The 3 months follow up session was powered to 80% to detect a change of 1m/s in CF-PWV compared to the baseline with a significant level of 5%. This

was based on the previous studies conducted in our centre which demonstrated average CF-PWV amongst pre dialysis CKD patients to be 9±1.6 m/s.

6.4 Results

6.4.1 Baseline characteristics

Patient's demographics and cardiovascular risk factors are listed in table 6.1.

Parameter	Patients (n=21)
Age(years)	69±9.5
Gender(male:female)	12:9
BMI	29±6.7
Ischemic heart disease	33%
Diabetes	33%
Hypertension	71%
Dyslipidaemia	71%
Smoking	57%
ACE inhibitors/ARB	71%
Pre eGFR (ml/min)	17±4
Albumin (g/l)	36±4
Hb (g/l)	11.4±1.4

Table 6.1 Demographics, CV risk factors and biochemical profile of the 3 months follow up participants.

6.4.2 Systemic haemodynamics and arterial stiffness

Preoperative, 2 weeks postoperative and the 3 month follow up measurements for the haemodynamic and arterial stiffness parameters are listed in table 6.2.

Parameters	Preoperative	2 weeks postoperative	3 months postoperative
Peripheral SBP	140±26	132±23	132±20
Peripheral DBP	75±12	66±11	63±10
Central SBP	130±24	120±22	120±21
Central DBP	73±12	66±11	64±11
Pulse pressure	57±17	55±17	56±17
CF-PWV (m/s)	12.45±2.6	10.8±2.4	11.0±2.8
AIx%	22±10	19±10	20±10
SV (ml)	115±32	120±40	126±37
HR (bpm)	60±12	63±10	62±10
CO (l/min)	6.3±1.4	6.9±1.2	7.3±1.3
TPR (mmHg.sec/ml)	1.0±0.23	0.84±0.20	0.8±0.15
EF (%)	45±14	51±12	53±11

Table 6.2 Preoperative, 2 weeks postoperative and the 3 month follow up measurements for the haemodynamics, left ventricular function and arterial stiffness parameters.

It can be concluded that the initial changes which was recorded 2 weeks postoperatively largely remains in the cohort with successful AVF formation even after 3 months. For instance, on comparing CF-PWV, 2 weeks and three months postoperatively, it did not change significantly (0.2±1.7 m/sec, p=0.7, figure 6.1). It can also be appreciated that both peripheral systolic and diastolic BP did not change (-0.2±16 mmHg, p=0.9 and -2±8 mmHg, p=0.7 respectively).

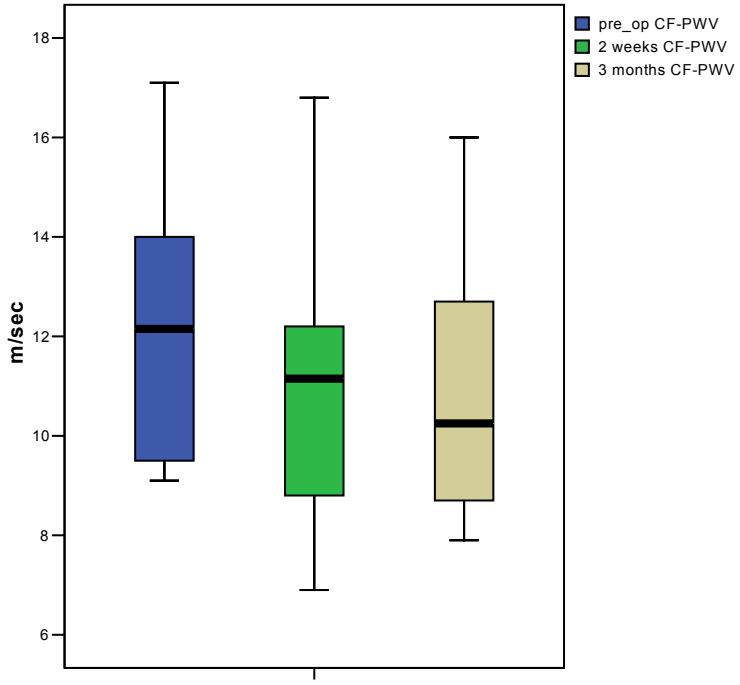

Figure 6.1 Carotid femoral pulse wave velocity (CF-PWV) 2 weeks and 3 months postoperatively.

Comparing the haemodynamic data between 2 weeks postoperative and 3 months postoperative readings, stroke volume (5.6±34 ml, p=0.5), HR (-2±7 bpm, p=0.3), TPR (-0.04±0.2, mmHg.sec /ml) and CO (0.4±1.8 l/min, p=0.36) did not change significantly.

6.4.3 Left ventricular ejection fraction

There is progressive increase in mean EF from preoperative measurements (table 6.2 and figure 6.1). Measurements taken 3 months postoperatively did not show significant change in the EF (3±7%, p=0.124) compared to 2 weeks postoperatively. None of the patients developed clinically overt high-output cardiac failure following the access operation.

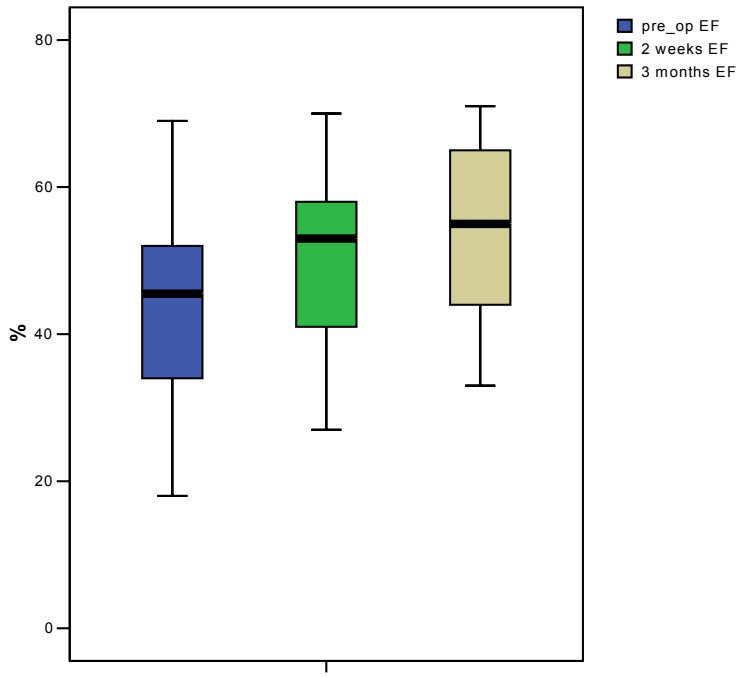

Figure 6.2 Left ventricular ejection fraction (EF) 2 weeks and 3 months postoperatively.

6.4.4 AVF Doppler ultrasound measurements

Preoperative, 2 weeks postoperative and 3 months follow up Doppler ultrasound measurements for the relevant blood vessels measurements (artery supplying the AVF and the draining vein), arterial and fistula blood flow are listed in table 6.3.

Parameters	Preoperative	2 weeks postoperative	3 months postoperative
Artery diameter (mm)	43±11	51±15	61±14
Acces vein	38±11	-	-
AVF diameter (mm)	-	66±17	83±21
Arterial blood flow (ml/min)	72±41	742±578	1176±603
AVF blood flow (ml/min)	-	1135±600	1652±941

Table 6.3 Doppler ultrasound measurements of diameter and blood flow in the AVF and the supplying vessels preoperatively, 2 weeks and 3 months postoperatively.

6.4.5 Body composition and laboratory data

There were no significant changes in total body water (intra and extra cellular water) and soft tissue composition after 3 months of AVF formation. Renal function as measured by eGFR was stable (17±5 ml/min) and comparable to preoperative (16±4 ml/min) and 2 weeks postoperative measurements (16±5 ml/min).

6.5 Discussion

This results chapter demonstrate for the first time that the AVF creation is associated with haemodynamic changes which persist for up to 3 months postoperatively. In the previous chapters we described the acute haemodynamic consequences of AVF creation which included increase in HR, SV and CO and reduction in TPR and BPs. Furthermore, AVF creation resulted in significant reduction in arterial stiffness and increase in left ventricular EF.

This is the first study to prospectively investigate the observed effects in detail on arterial stiffness and other cardiovascular structure and function and follow up the same cohort for up to 3 months. Previous studies varied in their follow up periods from days[140 147] to the maximum of 6 weeks[89] with small number of participant (n=9-17) or only focusing on studying a limited number of variables at a time. Furthermore, not all of the studies investigated CKD patients in predialysis stages. Uniquely, we have investigated the changes in systemic haemodynamics specifically in pre-dialysis population, without many of the confounders that would be involved in using a dialysis based population (changing volume status, resolution of uraemia, medication changes etc.). It has to be noted that these comparison studies were done on a smaller cohort of patients (n=21) compared to the original study.

Blood pressure remained lower than pre dialysis readings as were CF-PWV and AIx. The slight increase in CF-PWV and AIx may be attributed to natural

progression of disease in CKD patients; however; the changes were not statistically significant.

There was also slight increase in CO (0.4±1.8 l/min, p=0.36) and left ventricular ejection fraction (3±7%, p=0.124) but again they did not reach statistical significance.

The view that higher flow within the AVF is associated with high output cardiac failure is widely held. However this assertion has not been previously tested in prospective rigorous study. As mentioned previously, all patients who had a successful AVF formed showed an increase in their CO, however there did not appear to be any relationship between higher Qa values and CO. There was no clinical evidence of cardiac decompensation, with no change in total or ECW water in any of the patients studied. Echocardiography data demonstrated that even two weeks postoperatively, there was a significant increase in EF. This is similar to changes noted in previous studies[140 147]. The observed change in EF continued to be persistent up to three months postoperatively when the vascular access had matured.

6.5.2 Conclusions

Formation of an AVF resulted in a significant and persistent reduction in BP and arterial stiffness. There was increased HR and SV and reduced TPR. As a result, CO increased. Left ventricular EF also increased and this increase persisted up to 3 months postoperatively.

Overall the longer term post AVF adaptations might be characterised as potentially beneficial and this could well contribute to reduction in all cause and cardiovascular mortality in this group of patients compared to patients with other types of vascular access.

Chapter 7

Effects of AVF creation on local and systemic microcirculation

7 Results: effects of AVF creation on local and systemic microcirculation

7.1 Introduction

Little is known about the effect of AVF creation on local and systematic microcirculation. This chapter describes a study designed to characterise this in a cohort of predialysis population.

Endothelium is the largest organ in the body. It forms an interface between blood stream and vascular smooth muscle cell in the vessel wall. It responds to mechanical factor such as shear stress and pressure and humoral factors. Amongst its important functions are regulation of vasodilatation/vasoconstriction response, antiinflammatory and antithrombotic properties. Therefore, endothelial dysfunction is characterised by impairment in vasodilatory response and a proinflammatory and prothrombotic state.

Endothelial dysfunction has been associated with large number of cardiovascular risk factors and diseases including diabetes mellitus[165-167], hypertension[168-172], ischaemic heart disease, peripheral vascular disease, hypercholesterolemia, hyperhomocystinaemia and chronic kidney disease[173-174].

Several mechanisms have been proposed for endothelial dysfunction in these conditions including reduced bioavailability of nitric oxide (NO), increased asymmetric dimethylarginine (ADMA), increased oxidative stress, insulin resistance and accumulation of advanced glycation end product (AGE).

Defective microcirculatory perfusion of the myocardium in combination with the dialysis induced circulatory stress appears to be a significant component of the prevailing pathophysiological processes increasing the risk of myocardial hypoperfusion. Studies of myocardial blood flow during HD in patients with normal coronary angiograms[152] and dialysis induced cardiac segmental ischaemia in children on haemodialysis[153], have highlighted the existence of demand myocardial ischaemia in the absence of atheromatous large vessel coronary artery disease .

The assessment of subcutaneous dermal capillaries is a primary method for the assessment of the microcirculation. These vessels are representative of the microvascular supply to the heart [113] and kidneys [114]. This might allow study of the relatively accessible microcirculation to provide a window to critical central vascular beds. Abnormalities of endothelial function demonstrated using LDF can provide additional quantification of CV risk when used in conjunction with conventional risk scores [115]. LDF also appears able to document endothelial cell dysfunction in ESRF patients prior to clinical evidence of cardiovascular disease or diabetes mellitus [175].

Although some data are available on microcirculatory function in HD patients to date there are no studies examining the impact of vascular access formation either in the subtended distal limb or potential systemic effects that

might be important in the pathophysiology of the aggravated cardiovascular risk that patients on HD are subjected to.

7.2 Methods

7.2.1 Patients

Recruitment of patients are covered in chapter 4, section 4.2.1. and will not be discussed here again.

On the study days, patients were asked to refrain from taking caffeine containing drinks or smoke for at least 12 hours, and not to take their vasoactive medications for at least 24 hours before the study. Patients were allowed to acclimatise in the sitting position for at least 20 minutes prior to starting the study. All studies were done in a temperature and light controlled room (23°C).

All the studies were done on the palmar surface of forearm bilaterally. The arm was positioned at heart level and immobilized using a vacuum pillow containing polyurethane beads, which moulds to the shape of the arm (Germa, Sweden). To standardise the measurements further, two areas on the volar aspect of forearm were chosen, one for each drug. These were gently cleaned with alcohol wipe and sufficient time was allowed for the alcohol to fully evaporate. Locations on the forearm were chosen to avoid large vessels, pigmentations, scars and uneven surfaces. The drug delivery electrode chamber for acetylcholine iontophoresis was attached 5 cm below the elbow line and for sodium nitroprusside 10cm below the elbow line. The electrode chambers were covered with a plastic top to prevent leakage of

drugs. Baseline images, during which no drug was administered, were required for 90s prior to starting iontophoresis.

7.2.2 Iontophoresis

Anodal iontophoresis was used to deliver Ach and cathodal iontophoresis for SNP delivery using a battery powered iontophoresis controller (PeriIont 382, Perimed AB, Stockholm, Sweden). DC current of 0.02 mA was used for 420 seconds for iontophoresing both drugs. This protocol was used to specifically minimise non-specific vasodilatation by limiting the current density and charge density, and at the same time allowing enough time to obtain a maximum blood flow response to both drugs[176].

7.2.3 Laser Doppler perfusion imaging

For details of this technique, please see chapter 3, section 3.8. A laser Doppler perfusion imager (PIM II®, Perimed, Sweden) was used to measure changes in skin perfusion during iontophoresis. Particular attention was given to making sure that the distance between the head of the laser Doppler imager and the skin was constant at 15cm, and that it was parallel to the skin surface in all experiments.

Data was captured using the LDPIwin Version 2.6 software (Perimed AB, Sweden). The perfusion response is measured in arbitrary perfusion units (PU). As the PU values are

not absolute blood flow, the percentage change from baseline and the time taken to reach the maximum response were recorded.

Our group have previously shown the intra-individual coefficient of variation of this technique to be 18.7% for ACh and 15.2% for SNP[177]. Acetylcholine (Ach) and sodium nitroprusside (SNP) were used as endothelial dependant (ED) and non endothelial dependant (NED) vasodilators respectively. These were dissolved in deionised water to give a final concentration of 1%.

7.2.4 Statistical Analysis

Results are expressed as mean ± SD if parametric or median (interquartile range, IQR) if non-parametric unless otherwise stated. For comparing pre- and post operative data, either paired *t*-test or Wilcoxon rank sum test was used depending on normality of the distribution. An alpha error at $P<0.05$ was judged to be significant. All statistical analysis was performed with the use of the SPSS software package, version 12 (SPSS Inc., Chicago, IL, USA).

7.3 Results

7.3.1 Baseline characteristics

30/43 patients had a successful AVF operation (5 wrist and 25 elbow). Patients with failed AVF (13/43) procedures were utilised as sham operated controls. All patients had an end-to-side anastomosis and the operations were done as a day case under local anaesthetic. One patient died before attending the second study session. Two patients who had an unsuccessful

AVF operation first time, were re-recruited and consequently had a successful AVF formed.

Demographics, biochemical and cardiovascular risk factors of the study participants according to their operation outcome is discussed in chapter 4, section 4.4.1 and table 4.1.

Patients with failed AVF (13/43) procedures were utilised as sham operated controls. For the haemodynamic profile of both groups pre and postoperatively, please refer to table 4.2 (page68).

7.3.2 Baseline measurements

7.3.2.1 Patients with successful AVF operation

Preoperatively, in the successful group, baseline perfusion measurements in the forearm designated for AVF formation were NED 0.67±0.1 PU, and ED 0.62±0.07 PU. In non AVF forearm these figures were NED 0.64±0.08 PU and ED 0.6±0.05 PU.

Postoperatively, baseline readings in the AVF forearm of the successful group were NED 0.68±0.1 PU and ED 0.66±0.1 PU. In non AVF forearm these figures were NED 0.67±0.1 PU and ED 0.63±0.09 PU.

7.3.2.2 Patients with unsuccessful AVF operation

Preoperatively, baseline perfusion measurements in the forearm designated for AVF formation were NED 0.69±0.09 PU and ED 0.62±0.1 PU. In the non AVF forearm these figures were NED 0.66±0.1 PU and ED 0.59±0.1 PU.

Postoperatively, baseline readings in the attempted AVF forearm were NED 0.68±0.1 PU, ED 0.65±0.2 PU and in non AVF forearm NED 0.63±0.1 PU and ED 0.57±0.06 PU.

With the exception of baseline measurement for postoperative ED vasodilatation ($p=0.049$) in the non AVF forearm, there was no other statistically significant difference in the mean perfusion in baseline measurements between the successful and unsuccessful group both pre and postoperatively.

7.3.3 Maximum vasodilatation response to iontophoresis

7.3.3.1 Patients with successful AVF operation

Preoperatively, percentage of change from baseline perfusion in response to maximum vasodilatation in AVF forearm were NED 1.13±0.28 PU (68±33%) and ED 1.34± 0.34 PU (114±47%). For non AVF forearm these figures were NED 1.2± 0.27 PU (92±49%) and ED 1.32± 0.3 PU (118±54%).

Postoperatively, percentage of change from baseline perfusion in response to maximum vasodilatation in AVF forearm for NED 1.07± 0.3 PU (58±40%) and for ED 1.2± 0.3 PU (84±43%). For non AVF forearm, NED was 1.11± 0.3 PU (69±41%) and ED 1.27± 0.3 PU (105±65).

7.3.3.2 Patients with unsuccessful AVF operation

Percentage of change from baseline perfusion in response to maximum vasodilatation in the forearm designated for AVF formation were NED 1.28± 0.3 PU (88±64%) and ED 1.45± 0.4 PU (140±76%). In the non AVF forearm these figures were NED 1.27± 0.3 PU (93±45%) and ED 1.47± 0.4 PU (158±90%).

Comparing the maximum vasodilatation between successful and unsuccessful groups pre operatively, there was no significant difference in either forearms for both ED and NED vasodilatation.

Postoperatively, percentage of change from baseline perfusion in the forearm with attempted AVF formation were NED 1.28± 0.3 PU (89±43%) and ED

1.56± 0.6 PU (135±46%). For non AVF forearm these figures were NED 1.34±0.33 PU (114±59%) and ED 1.33± 0.4 PU (133±67%).

When comparing maximum vasodilatation between the successful and unsuccessful groups postoperatively, maximum response to ED vasodilatation was significantly different ($p=0.02$) in the AVF forearm. In addition, postoperative maximum response to NED vasodilatation was significantly different ($p=0.01$) in the non AVF forearm between the two groups.

The time required to achieve maximum vasodilatation was not statistically different between the groups for corresponding tests in the corresponding limbs either pre or postoperatively.

When pre and postoperative measurements are compared, a reduction in maximum ED vasodilatation response to Ach was observed in the fistula forearm in patients with successful AVF formation (-36±46%, $p<0.001$, see figure7.1). This did not change significantly in the non fistula arm of the successful AVF group.

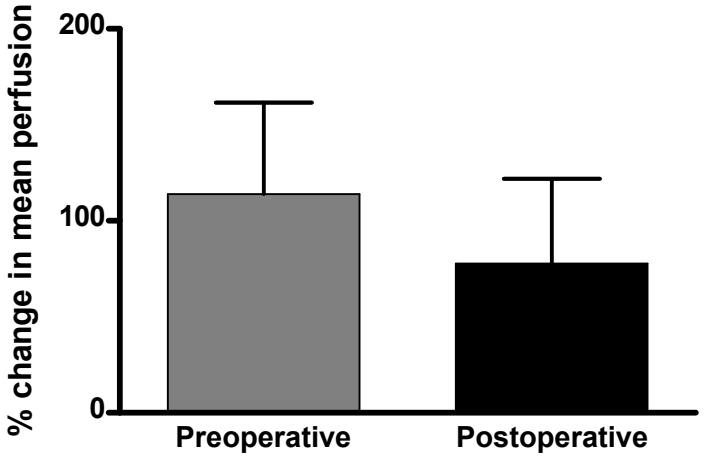

Figure 7.1 Percentage reduction in mean perfusion in AVF arm in successful group in response to Ach iontophoresis.

Similarly, maximum NED vasodilatation response to SNP was reduced in the non fistula forearm in patients who had a successful AVF formation (-23±40%, p=0.01, see figure 7.2). Again, this change was not noted in the AVF forearm. Patients who had unsuccessful AVF operation did not show any statistically significant change in their ED and NED vasodilatation response between pre and post operative measurements.

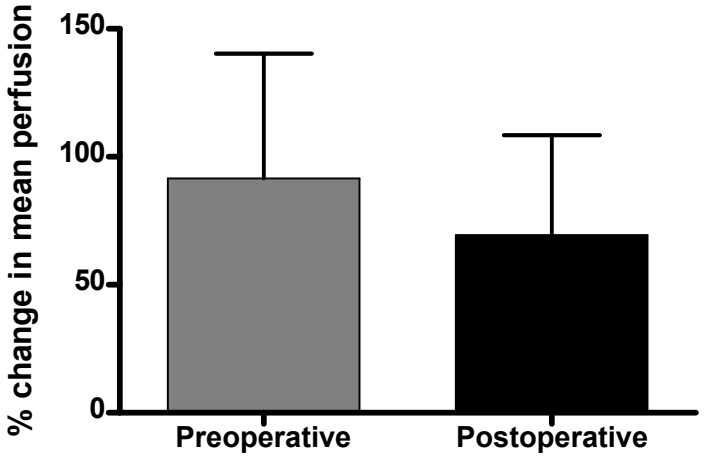

Figure 7.2. Percentage reduction in mean perfusion in non AVF arm in successful group in response to SNP iontophoresis.

We also looked into the effect of the AVF location on the described results. We subdivided the patients who had successful AVF created according to the location of their AVF into wrist and elbow group. There was no statistically significant difference in the baseline and maximum vasodilatation response pre and postoperatively between wrist and elbow AVFs.

7.4 Discussion

This is the first study to prospectively investigate the microvascular effect of AVF formation locally and systematically. The utilisation of patients with primary technical failure provides the opportunity to study those effects controlled against a group of patients undergoing the same progression of uraemic factors, but without a formed shunt.

We have demonstrated for the first time that AVF formation is associated with reduction in endothelial dependant vasodilatation in the fistula forearm.

Furthermore, it had systemic effect by reducing non endothelial dependant vasodilatation in the contralateral forearm.

The presence of endothelial dysfunction is well described in patients with different stages of CKD. Thambyrajah et al.[173] found that in comparison to healthy controls, patients with various abnormal glomerular filtration rate showed abnormality of endothelial dependant but not non endothelial dependant vasodilatation even in absence of atherosclerotic cardiovascular disease. Farkas et al. [178] showed impaired ED and NED vasodilatation in hypertensive haemodialysis patients using laser Doppler flowmetry and iontophoresis of Ach and SNP. Their findings were also supported by alteration in levels of endothelial function biomarkers in this group of patient.

We have demonstrated for the first time that AVF formation affects microcirculation function both locally and systematically. Alteration in pressure and shear stress (the frictional force generated from blood flow on the vessel wall) on vascular endothelium layer can contribute to these changes. Change in shear stress has been associated with modifying physiological and pathological processes associated with endothelium such as vasodilatation/vasoconstriction, endothelial cell proliferation, inflammation and thrombosis. It is well recognised that in normal blood vessels, the shear stress generated by normal laminar flow results in continuous production of nitric oxide (NO) from the endothelial cells which in turn protects the cells from apoptosis[179-180]. Furthermore, it has been proposed that sites with turbulent blood flow and altered shear stress are associated with increased

cell turnover[181-182]. The association between abnormal turbulent blood flow and change in endothelial cell shape and function has also been well described[183-186]. Certainly, creation of an AVF generates turbulent blood flow in local circulation. It will also bypass the normal arteriole-capillary-veniole blood flow pathway and generates a shunt with flow rates varying between 500ml/min to even more than 3000ml/min. Furthermore, it has been shown that both wall shear stress and vessels diameter increased suddenly in the proximal arteries after AVF formation and this increase in wall shear stress remained unchanged 1 year after the operation[187].

Moreover, we have already demonstrated that AVF creation is associated with marked functional and structural cardiovascular changes[188]. These changes included increase in cardiac output, reduced total peripheral resistance, reduced systolic and diastolic blood pressures, increased ejection fraction and a reduction in the markers of arterial stiffness.

We propose that the observed changes in microcirculation, both locally and systemically are resulting from firstly the changes to function and structure of the endothelium induced by alteration in the vascular shear stress locally and secondly wider systemic haemodynamic effects caused by the AVF formation.

The limitations of this study are small sample size and lack of concurrent measurement of wall shear stress. This is the first study looking prospectively into microcirculatory changes/endothelial function in this group of patients.

For the sake of time and decreasing the demand on the participants, measuring wall shear stress was not performed

It is currently not known how these changes in microcirculation affect other organs blood flow and pathophysiology. We have identified that both systemic and local vasodilatation responses change after AVF creation. From our study, one interesting negative finding was lack of correlation between baseline and maximum vasodilatation and the likelihood of an AVF formation being successful or not. However; endothelial dysfunction may well contribute to further impairment of already diseased peripheral vessels in CKD patients and may well play an important role in future local vascular complications and AVF failure. Certainly further prospective studies are required to explore the relationship between microcirculation and fistula dysfunction.

Systemically, it is currently unknown how this reduction in NED vasodilatation secondary to AVF formation affects cardiovascular performance and prognosis in this group of patients. Previous studies have indicated that peak vasodilatation response was significantly reduced in a cohort of end stage renal disease patients at high cardiovascular risk measured using Framingham and Cardiorisk scores[115]. It is not currently known if this further reduction in vasodilatation response secondary to AVF formation is associated with an increase in cardiovascular mortality and morbidity and again it requires further study.

Given such a high rate of cardiovascular mortality and morbidity in our CKD patients, studying these effects will certainly increase our understanding

about the scope of problems encountered by patients, may help in developing new therapies to improve outcome in CKD.

Chapter 8

Higher arteriovenous fistulae blood flows are associated with a lower level of dialysis induced cardiac injury

8 Results: higher arteriovenous fistula blood flow are associated with a lower level of dialysis induced cardiac injury

8.1 Introduction

Very high fistula flows (>1500ml/min) are generally recognised as being capable of inducing a high output state and precipitating cardiac failure[89 139]. However, high fistula blood flow (Qa) does not seem to predispose to increase mortality and indeed a reduced Qa might be associated with decreased survival[189].

It is well recognised that dialysis patients display hugely elevated rates of cardiac mortality. It is also becoming appreciated that this rate of cardiovascular attrition is not driven by the same variety of risk factors, or pathophysiological processes that are important in the general population[142].

Cardiac failure develops in as many as 25-50% of HD patients and confers a dramatic reduction in probability of survival[143]. In addition, a significant percentage of cardiac mortality is due to sudden death[190-191]. Abnormal ventricular morphology and function appear to be major determinants of cardiovascular outcome in this patient group[192].

It has long been suspected that myocardial ischaemia may be precipitated by HD. There have been a variety of studies confirming dialysis induced myocardial ischaemia[192-193]. In conjunction with this, HD patients are particularly susceptible to myocardial ischaemia. In addition to the high

prevalence of coronary artery atheroma[144], diabetic dialysis patients have been shown to have a reduced coronary flow reserve (CFR) in the absence of coronary vessel lesions[145]. Haemodialysis patients characteristically also exhibit left ventricular hypertrophy (LVH), reduced peripheral arterial compliance, impaired microcirculation[107 194] and ineffective vasoregulation (in the face of HD coupled with ultrafiltration). All of these factors predispose to demand myocardial ischaemia.

In patients with coronary artery disease, but without CKD, transient myocardial ischaemia may lead to left ventricular dysfunction that can persist after the return of normal perfusion. This prolonged dysfunction is known as myocardial stunning[195]. Repetitive episodes of ischaemia can be cumulative and have been shown to lead to prolonged left ventricular dysfunction. Myocardial stunning is thought to be a causative mechanism for heart failure.

We have demonstrated that the phenomenon of dialysis induced cardiac injury is common, occurring in around 60% of patients studied, and is associated with global and segmental significant reductions in myocardial blood flow[152] leading to a reduction in the left ventricular ejection fraction, increased arrhythmias and decreased survival[154 196]. Dialysis induced myocardial stunning is partially abrogatable by modification of the dialysis process to improve the systemic haemodynamic response to treatment[197-198].

The aim of this study was to examine the effect of local AVF blood flow rate on dialysis induced, ischaemic based, reversible cardiac injury. Thus determining whether or not AVF formation was likely to favour the

development of demand myocardial ischaemia, or potentially provide a degree of cardio-protection.

8.2 Methods

8.2.1 Patients

Patients within this study represent the 50 patients with mature fully functional native vascular access within a larger group of seventy prevalent HD patients. These were recruited for a 12-month observational cohort study from a single hospital based haemodialysis unit. Patients were excluded if they had pre-existing severe LV systolic dysfunction (NYHA IV) or inadequate echocardiographical windows to obtain images of sufficient quality. Only one patient was excluded on this basis. There were no other significant exclusion criteria. All patient ages and primary aetiologies of CKD were included.

All patients were on thrice weekly dialysis lasting four hours. Patients were divided into tertiles based on Qa value (summary of patient characteristics is given in table 8.1).

All patients gave informed consent before the study start, and ethical approval for the project was granted by Derbyshire Local Research Ethics Committee.

	Qa<500 ml/min	Qa500-1000 ml/min	Qa>1000 ml/min	P value
Qa (ml/min)	291±101	739±130	1265±221	<0.001 (between all values)
N	19	15	16	
Age (years)	64.1 ± 13.4	62.3 ± 11.2	65.1 ± 11.9	Ns
Dialysis vintage (months)	46.8 ± 28.7	47.6 ± 24.3	50.6 ± 31.3	Ns
Sex (m:f)	14:5	9:6	9:7	Ns
Diabetic (%)	21	33	25	Ns
Previous CV Co-morbidities (%)	16	38	31	Ns
Hb (g/dl)	10.8± 0.6	10.6± 0.4	11.1± 0.6	Ns
Corrected Calcium (mmol/l)	2.39± 0.13	2.44± 0.11	2.41± 0.12	Ns
Phosphate (mmol/l)	1.59± 0.9	1.61± 0.9	1.60± 0.08	Ns
Albumin (g/l)	33.6± 5	34.1± 4.9	34.3± 5	Ns
cTnT (µg/l)	0.12±0.02	0.09±0.03	0.03±0.01	0.01
Kt/V_{UREA}	1.48± 0.3	1.46± 0.4	1.52± 0.3	Ns
Interdialytic weight gain (kg)	1.65± 0.2	1.72± 0.3	1.49± 0.2	Ns
Systolic BP (mmHg)	149 ±28	144 ±24	138 ±23	Ns
Diastolic BP (mmHg)	80 ±15	77 ±12	76 ±13	Ns
Pulse pressure (mmHg)	67 ±21	65 ±23	64 ±21	Ns

Table 8.1 Description of the study subjects categorised by Qa value

8.2.2 Study design

Patients underwent Qa measurement 30 minutes into the first HD session after the short interdialytic interval. Measurement of Q_A was performed utilising a non operator dependant technique based on dual measurement of ionic dialysance and flow reversal (using specific crossed line extensions and clamps, to avoid the need to disconnect blood lines)[199]. All Qa studies were performed within one week of the echo based study visit. This is to avoid the possibility that the Qa measurement procedure (with period of fixed UF rate) did not impact on any possible observed changes on myocardial stunning.

Blood samples were collected before each session in lithium heparin and EDTA tubes, and biochemical analysis performed on a multichannel autoanalyser. Cardiac troponin-T (cTnT) analysis was performed using a third generation electrochemiluminescence assay (Roche diagnostics, Lewes, UK).

8.2.3 Echocardiography

Two-dimensional echocardiography was performed serially throughout dialysis sessions by using commercially available equipment (1.5-3.6 MHz 3S probe, Vivid 3®, GE medical systems, Sonigen, Germany). A single experienced technician carried out all measurements with the patients in the left lateral position. Images were recorded before starting dialysis (baseline), and 240 minutes during dialysis. Standard apical two-chamber and four-chamber views (to visualize the LV endocardial border in two planes at 90° to each other) were digitally recorded onto a compact disc for offline analysis.

Digital images were subsequently transferred and analysed by using a personal computer–based digitising program (CMS-View, DICOM review station and Echo-CMS; MEDIS, Leiden, The Netherlands), as previously described [200]. Three consecutive heartbeats were analysed for each time point (extra-systolic beats were excluded). Endocardial borders (excluding papillary muscles) were traced semi-automatically for each video frame of the 3-beat sequence, and any anomalies were corrected for manually. Maximal displacement of the endocardial border from a centre point then was measured over each of 100 chords around the left ventricular (LV) wall, corrected for end-diastolic LV circumference, and expressed as percentage of shortening fraction (SF). Severity of dialysis induced myocardial stunning was represented by the number of segments that displayed significant reduction in SF, and the mean cumulative reduction in SF in those areas.

Each apical view was divided into 5 segments, and SF for the chords in each segment was averaged so that 10 regions of the left ventricle were assessed at each time. New regional wall motion abnormalities (RWMA) were defined as segments that showed a decline in SF greater than 20% from baseline.

Left ventricular ejection fraction (LVEF) was calculated using LV volumes at end systole and end diastole, measured by the biplane disk method. LV mass index (LVMI) was calculated from each patient's original baseline images using the Devereux formula corrected for height.

8.2.4 Haemodialysis details

Dialysis was performed using Hospal Integra monitors (Hospal, Mirandola, Italy) using low-flux polysulfone dialysers, either 1.8 or 2.0m^2, per individual patients' usual prescriptions (LOPS 18/20; Braun Medical Ltd, Sheffield, UK). Dialysate fluid contained sodium, 138 mmol/L, potassium 1 mmol/L, calcium 1.25 mmol/L, magnesium 5 mmol/L, bicarbonate 32 mmol/L, glucose 1 g/L, and acetate 3 mmol/L.

All treatments were of four hours' duration, and anticoagulation was with unfractionated heparin. Dialysate flow was 500 mL/min, and dialysate temperature was set at 36.5°C. For each session, net fluid removal was set on an individual basis according to ideal dry weight. Blood pump speed varied between 250 and 450 mL/min, depending on the patient's vascular access.

8.3 Statistical analysis

Results are expressed as mean ± SD for parametric data or median (interquartile range, IQR). Echocardiographic, BP and haemodynamic data were analysed using one-way analysis of variance (ANOVA) with a design for repeated measures and Bonferroni's test to correct for multiple comparisons. Chi squared tests were used to compare categorical data. For other data, either the paired *t*-test or Wilcoxon rank sum test was used depending on normality of the distribution. An alpha error at $P<0.05$ was judged to be significant.

8.4 Results

8.4.1 Qa values and patient characteristics

The patients were divided into three groups on the basis of their Qa values. The values were based upon current K/DOQI recommendations for vascular access monitoring (concerning recommendations for enhanced access monitoring and direct vascular imaging). The mean Qa in the lower access flow group was 291±101 ml/min vs. 739±130 ml/min and 1265±221 ml/min in the higher flow groups (p= 0.001 between all values). Even in the highest flow group the maximum Qa was 1881 ml/min. 65% of AVFs were radiocephalic, with the balance being brachiocephalic (apart from two AVF utilising the basilic vein). There were no differences in the distribution of AVF type between the patient groups. 90% of these AVFs had been formed with a common approach of local anaesthesia and a side to side anastamosis, with no formal surgical attempt at flow assessment or limitation. All AVFs had been *in situ* for at least six months.

There were no statistically significant differences between the patients in these groups for any of the routine patient descriptors, standard haematology/ biochemical testing, delivery of dialysis, nutrition (serum albumin), blood pressure (BP) or pulse pressure (summarised in table 8.1). Detailed study of intradialytic blood pressure was beyond the scope of this study, but there were no statistically significant differences between pre and post dialysis systolic or diastolic BP.

8.4.2 Echocardiographic assessment

There were no significant differences in systolic function, as assessed by resting LVEF between the three groups (table 8.2). Patients with higher Qa's displayed a trend towards lower LVMI (82.1±17, 78±18 and 72±21 ml/min respectively for the low to high Qa patient groups). This did not reach statistical significance (p = 0.065 between Qa <500 ml/min and >1000 ml/min patient groups).

	Qa < 500 ml/min	Qa 500-1000 ml/min	Qa >1000 ml/min	P value
LVEF (%)	56± 12.1	54± 11.7	56± 11.4	Ns
LVMI ($g/m^{2.7}$)	82.1±17	78±18	72±21	Ns
Number of segments significant reduction in SF (per patient)	3.8± 2.6	3.4± 2.1	2.3± 2.0	Ns
Sum of reduction in fractional shortening in effected segments (per patient)	-187± 161‡	-160± 102	-101± 102‡	0.02

Table 8.2 Echocardiographic results for patients divided into tertiles on the basis of Qa.

When considering the presence or absence of left ventricular hypertrophy, there was a significantly lower proportion of patients when divided into two groups (22/29 patients, 76% vs. 8/14 patients, 55% p=0.01) (figure 8.1).

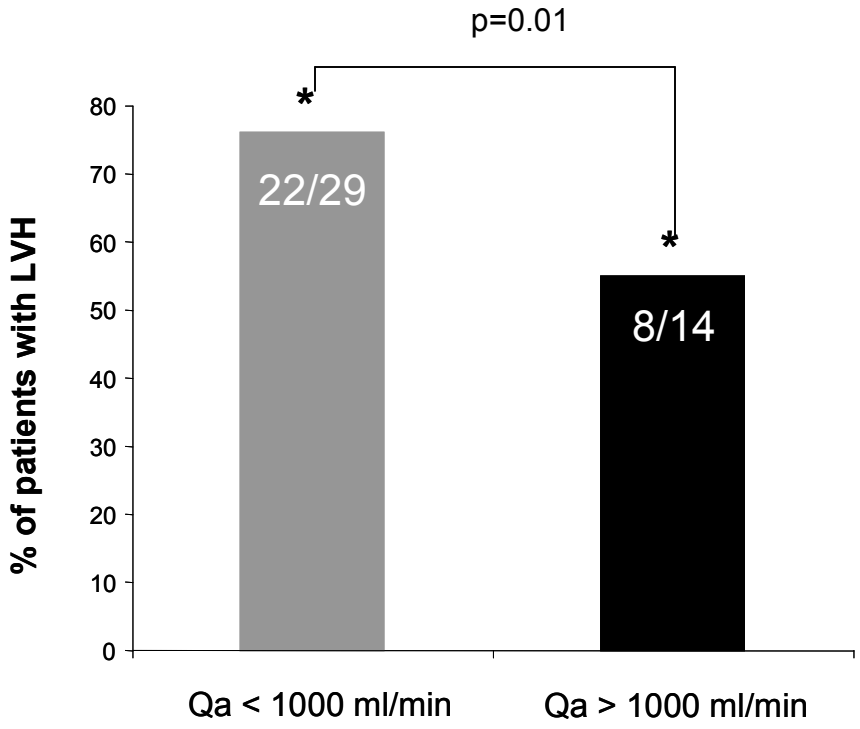

Figure 8.1 Proportion of patients with LVH characterised by their Qa

Patients with higher Qa AVFs were subject to significantly less dialysis induced acute cardiac injury. This was assessed by both the number of myocardial segments that developed a reduction in contractile function (RWMAs), and the sum of reduction in fractional shortening in those affected segments. Number of RWMAs induced by HD was sequentially lower in the patient groups with higher flow AVFs (3.8± 2.6, 3.4± 2.1 and 2.3± 2.0

respectively for the low to high Qa patient groups). This did not reach statistical significance (p = 0.07 between Qa <500 ml/min and >1000 ml/min patient groups).

There was however a statistically significant reduction in sum reduction in fractional shortening in patients with higher Qa AVFs (-187± 161, -160± 102 and -101± 102% respectively for the low to high Qa patient groups, p=0.02). These differences in function were reflected by similar differences in biochemical evidence of cardiac injury (pre dialysis serum cTnT values 0.12±0.02, 0.09±0.03 and 0.03±0.01 µg/l respectively for the low to high Qa patient groups). This reached a statistical significance of p=0.01 between Qa <500 ml/min and >1000 ml/min patient groups. Differences between both extremes and the Qa 500-1000 ml/min group were both p=0.06.

Stepwise multivariate analysis of those factors contributing to the presence of RWMAs revealed that the following were all independent variables associated with the development of HD-induced RWMAs: maximum reduction in SBP, per mmHg (OR 1.3, CI 1.04-1.2, P=0.001); UF volume per litre (OR 4.2, CI 1.42-21.1, P=0.01), cTnT concentrations per µg/L (OR 1.08, 1.01-1.19, P=0.03) and Qa with a reduction in OR for higher flow AVF vs. lower and medium flow of 0.76 (CI 0.51-0.97, P=0.03) (Nagelkerke R^2=0.48 of the model overall). All other factors, including diabetes mellitus, albumin levels and IHD did not enter the final analysis.

8.5 Discussion

This study is the first to provide preliminary evidence that AVF function, and its effect upon demand myocardial ischaemia, might play a role in the observed improved longer term outcomes in patients receiving dialysis via native AVFs.

There are no consistently identified factors that determine Qa within an AVF in an individual patient. Although various studies have identified cardiac index, AVF position, ischaemic based primary renal disease[201] and systolic blood pressure[202] as potential factors, we found no significant differences in these, or any other patient descriptive factors, between the various Qa groups. The absolute flows within this study are in keeping with those generally described, but with a noted absence of many very high flow AVFs (Qa>1500 ml/min) reducing the likelihood of high output cardiac failure [203]. It did not appear that by separating patients into groups based on Qa prior to analysing them for cardiac injury, we were generating three patient groups likely to have a differing response to cardiac stress unrelated to AVF function. There were no differences between the groups for overt cardiovascular disease, age, diabetes, blood pressure or resting LVEF.

We have previously identified dialysis induced myocardial stunning as a common consequence of HD[197-198 204-205], and identified that it is associated with a number of dialysis related factors (ultrafiltration volume and maintenance of BP primarily). Patients in this current study, despite exhibiting differing degrees of new RWMA development with dialysis, were not different

with respect to those factors. Access type and function had not been a feature of the previous studies. Multivariate analysis has confirmed the crucial role played by dialysis induced relative hypotension and UF volume/rate that we have previously reported[22]. In addition higher flow AVFs appear to be independently associated with a lower degree of dialysis induced cardiac stunning. The small numbers in these sub groups though are not ideal for such multivariate data treatment.

We did not attempt to study the effect of not having an AVF at all largely for logistical reasons. Within our renal service only a small proportion of patients (10-15%) rely on central catheter access. These patients are characterised by being new to dialysis or having severe co-morbidities or acute intercurrent illness. The virtual absence of patients with arterio-venous grafts (AVG) within our unit precluded inclusion of this patient group, and must be considered a limitation of this initial study. The lack of of patients within this study with AVGs mean that no comment can be made concerning the potential impact of this form of vascular access.

The literature concerning the cardiovascular results of AVF formation is both limited and short term. Although there is some evidence that formation of an AVF might predispose to cardiac ischaemia and LVH development, there are no data directly relating to the degree of flow in the AVF (well summarised in review by McRae and co-authors)[206]. Although there are currently very few studies directed at the systemic cardiovascular consequences of AVF formation and function it is intriguing to speculate on what might be the

mechanism for the observed cardio-protective effect, from a well functioning AVF. Possibilities might include alteration of arterial stiffness (which can regulate the likelihood of demand myocardial ischaemia[207], alteration of sympathetic over activity, reduction in either pre load or after load and the delivery of greater amounts of oxygenated blood to the central circulation (with modulation of central baroreflex control and coronary perfusion). The finding that patients with higher flow AVFs appeared to have developed less increase in LV mass suggests that alteration in arterial compliance, or ventricular loading may indeed be involved.

Of particular interest might be the role of remote ischaemic preconditioning (RIPC). It has been appreciated for some years that transient ischaemia of a vulnerable organ provides a degree of protection for that organ in the face of a further ischaemic insult. It also appears that remote ischaemia (e.g. from intermittently cuffed limb ischaemia) results in a both a humoral and neuronal cascade, providing protection of the heart from subsequent ischaemic injury[208] . Intermittent upper limb ischaemia has been recently demonstrated to protect patients undergoing coronary artery bypass grafting from procedure related cardiac ischaemic damage (as evidenced by serum cTnT levels)[209]. Distal relative ischaemia from a higher flow AVF, either at rest, or during the intermittent use of the AVF during HD, might result in a similar process and provide a degree of RIPC for the heart.

In conclusion, this study provides initial data to suggest that a well functioning, higher flow AVF is associated with reduced incidence of dialysis

related cardiac ischaemia. It is consistent with the empirical clinical observation that patients with well functioning access appear to have better clinical outcomes, and provides additional reinforcement for current programs promoting the use and maintenance of definitive vascular access.

Chapter 9

Conclusions, Study Limitations and Future work

9 Conclusions, Study Limitations and Future Work

9.1 Conclusions

CKD is a major health problem worldwide with increasing incidence and prevalence. Cardiovascular diseases are a major cause for mortality and morbidity in CKD patients. It has been increasingly appreciated that the risk factors for cardiovascular diseases in CKD patients are beyond and above the traditional risk factors in non CKD patients. One of the important non traditional risk factor which has been associated with increased cardiovascular and all cause mortality in CKD population is increased arterial stiffness. In addition to these traditional and non traditional risk factors, these patients are exposed to complications from uraemia and dialysis treatment for their renal failure. As dialysis population continue to grow, this excess in mortality is becoming more of an important issue.

The type of access used to administer haemodialysis has been a controversial subject until recent years. Large studies have shown reduced morbidity (and hospitalisation) and mortality using native AVFs compared to both AVF grafts and tunnelled venous lines[164]. The reason behind improved survival in patients with native AVFs has always been attributed to reduced risk of infection associated with using AVFs. However, our results are first to suggest that there might be other factors which could potentially contribute to this improvement in survivals in patients with native AVFs.

Although different, limited aspects of haemodynamic changes in response to AVF creation has been looked into in different studies, no previous study has looked into the effects of AVF formation on arterial stiffness. Furthermore, our study prospectively followed up a cohort of predialysis patients immediately after AVF formation and after allowing sometime for the AVF to mature.

Our cohort consisted of CKD 4 and 5 patients attending low clearance clinic and were referred for AVF creation. The success rate obtained in this cohort generally mirrors average national success rate. One of the advantage of this study was using patients underwent unsuccessful AVF created as sham operated controls for comparison purposes.

Firstly, we looked into the acute effect of AVF formation on systemic haemodynamics. We demonstrated that AVF formation is associated with reduction in TPR, increased SV and HR. As a result of these changes, CO significantly increased. These findings were consistent with the findings from previous studies and only happened in patients with successful AVF s. Furthermore; analysis of our echocardiography data confirmed an increase in EF in patients who had successful AVF formed. There was no evidence of acute cardiac decompensation clinically in this group of patients.

Furthermore, both systolic and diastolic BP were both reduced after a successful operation. This was true for measurements done peripherally and centrally using aortic pulse wave analysis.

AVF formation was also acutely associated with a marked reduction in arterial stiffness as measured by CF-PWV and AIx. This was in contrast to previous

studies (n=9) measuring AIx (but not CF-PWV which is the gold standard) reporting no significant changes during their follow up period. More recently, Utescu *et al* demonstrated that successful AVF formation was associated with reduction in CF-PWV and mean BP and a non significant increase in AIx[159]. Interestingly, although baseline CF-PWV and the decrease in CF-PWV over 3 months are similar in our study to the study by Utescu *et al*, our present study showed that as markers of arterial stiffness, both CF-PWV and AIx were significantly reduced 2 weeks postoperatively and it persisted for 3 months afterwards. One of the crucial differences between the two studies is that none of our patients were commenced on haemodialysis treatment before the last study session. In contrast Utescu *et al* reported that 52% of their participants were already receiving HD at the beginning of the study and this increased to 71% at the end of the study.

As part of the analysis, we looked into the correlation between ∆PWV and other haemodynamic variables. The reason behind this is the proposed association between the change in vascular stiffness being caused by change in blood pressure. A stepwise multiple regression model showed that both changes in diastolic BP and presence of diabetes were independently associated with reduction of CF-PWV with reduction in diastolic BP independently contributing to 25% of the model. We suggested that although the observed change in the blood pressure contributes to reduction in PWV, there are other indeterminate factors largely responsible for this reduction. This is supported by previous studies which demonstrated that the lack PWV

reduction in response to BP reduction is an independent significant predictor of mortality in CKD patients.

We concluded that at least during the acute stages following AVF creation, the adaptive changes in cardiovascular physiology could be potentially described as beneficial in this group of patients as indicative by reduction in BP s and arterial stiffness. It is important to mention that these acute haemodynamic changes were observed without any noticeable change in body water content/soft tissue composition. Renal function and haemoglobin also remained unchanged postoperatively.

Investigating the longer term consequences of AVF formation was also planned when the study was designed. Therefore, those patients who had successful AVF formed and consented to undergo the second session, were re-studied in a similar way after AVF maturation (3 months postoperatively).

We demonstrated for the first time that the adaptive changes acquired immediately after the AVF formation, were persistent. CF-PWV and AIx both stayed reduced compared to their preoperative values. This was also true for the BP s which stayed low as well. Mean CO was slightly higher 3 moths postoperatively compared to the 2 weeks postoperative values as was left ventricular EF. Again, no patient suffered acute cardiac decompensation clinically during the second follow up period and no significant change in body composition, hydration status, renal function or haemoglobin was observed.

When we studied a different cohort of patients cross-sectionally, we demonstrated that dialysis induced myocardial stunning was reduced in

patients with higher AVF flows. These findings were consistent with the empirical clinical observation that patients with well functioning access appear to have better clinical outcomes. However; this study was limited by its design and further longitudinal studies are required to ascertain the findings.

As microcirculation is an important and integral part of cardiovascular physiology, we also looked into the acute effects of AVF creation on microcirculatory function. Changes in vasodilatation response to iontophoresis (both ED and NED) were measured pre and postoperatively, in the fistula forearm and non fistula forearm.

We discovered that although baseline readings were broadly similar in both groups for both arms, changes only happened in patients who had successful AVF s created. Significant reduction in maximum ED vasodilatation was observed in the AVF forearm when comparing the preoperative and postoperative values. In the non AVF forearm (systemically), a significant reduction in maximum response to NED was observed postoperatively compared to their preoperative values.

We suggested that the changes in AVF forearm could well be explained by changes in the local shear stress inducing secondary changes in endothelial function as a result of the AVF formation. Furthermore, systemically, the described changes in haemodynamics and arterial stiffness could well contribute to the described changes in microcirculation function in the non AVF arm.

Majority of patients with CKD have already on going endothelial dysfunction secondary to other metabolic and biochemical abnormalities, even prior to AVF formation. This change in endothelial function secondary to the AVF formation is an important observation due to the previous studies linking abnormal endothelial function with abnormalities of other vital circulation. One interesting negative finding was lack of correlation between baseline and maximum vasodilatation and the likelihood of an AVF formation being successful or not. However; endothelial dysfunction may well contribute to further impairment of already diseased peripheral vessels in CKD patients and may well play an important role in future local vascular complications and AVF failure. Certainly further prospective studies are required to explore the relationship between microcirculation and fistula dysfunction.

In conclusion, the results described in this thesis demonstrate that AVF formation is associated with significant changes in haemodynamics, left ventricular ejection fraction and arterial stiffness. These changes are persistent even after AVF maturation. Associated with these, was a significant reduction in maximum ED and NED vasodilatation response in the AVF and non AVF forearm respectively.

The findings of this thesis suggest that the improvement in the haemodynamic profile and arterial stiffness indices could well contribute to

improved overall survival of patients who have native AVF s as their primary and definitive vascular access compared to other vascular accesses types.

9.2 Limitations

This study has a number of limitations. Although this study is the first to prospectively follow a cohort of patients after their first AVF formation and extensively investigate the acute postoperative changes in their haemodynamics and after a reasonable period of maturation, we have not studied the longer term adaptive changes that might be associated with the AVF in use.

Due to low rate of AV graft placement in our centre, this study did not include any patient with AV graft. It is therefore unrealistic to comment on how the haemodynamic changes in response to a graft placement. Likewise we did not have access to a comparable population of well controlled patients within the low clearance clinic setting who were dialysed with tunnelled venous catheters.

Other potential limitations to this study include small number of the sample which is mostly due to the study design of only including patients without prior exposure to haemodialysis undergoing AVF formation. The small sample size hindered performing further analysis of the cohort according to their Qa flow or the type of the fistula constructed.

Similar to all studies measuring haemodynamics, each study session will be inevitably limited by its duration. Every care has been taken to recreate identical situations during each session. Furthermore, individual patient were studied during the same time of the day and in the same place to avoid potential external influences on repeated measurements.

The study sample was typical for CKD 4/5 in a hospital based low clearance setting, and clearly a degree of selection for patients potentially suitable for HD would occur. The small sample size hindered performing further analysis of the cohort according to their Qa flow or the type of the fistula constructed.

One of the other study limitations is the use of Doppler ultrasound to measure the flow in the AVF and the supplying artery. Doppler ultrasound is well established as a safe, non-invasive, and versatile diagnostic modality and is widely used to investigate problematic AVF s. However, as with any ultrasound technique, assessment of flow in the AVF remains subjective. To minimise these factors, a single operator acquired all Doppler images to minimise inter-observer variability. This has been further reduced by using a section of AVF devoid of turbulent flow and as straight as possible. Two measurements were taken and the average calculated and used for subsequent analysis. Nevertheless, Doppler ultrasound remains the only method to measure the flow rate in a fistula which has not been used for dialysis yet.

Despite all the described limitations, this study increases our understanding of the acute and longer term physiological changes in cardiovascular structure

and function associated with constructing an AVF. All of the assumptions made in the initial choice of the sample size with respect to the primary end point of change in PWV were met.

Assessing the effect of AVF flow rate on dialysis induced cardiac injury had a number of significant limitations. It only provides preliminary evidence in a cross sectional study of patients. Prospective investigation of the effects of AVF formation and systemic cardiovascular effects are imperative. The method of measuring Qa is performed during dialysis, and there might be artefacts from cardiac performance itself. There was however no difference in blood pressures between the three groups, and all Qa measurements were made in the first 30 minutes of dialysis to minimise this effect. Dialysis induced RWMA development does not usually become evident until at least 2 hours into HD[197]. The ionic dialysance method itself has been correlated well with ultrasonic saline dilution techniques[199], but may have a degree of variance in the absolute Qa values recorded. Addition of a further non HD based assessment (MR or ultrasound Doppler) would strengthen this element. The advantage of the ionic dialysance based method is that it removes much of the operator dependency of more commonly employed techniques, and is in general use for access surveillance within our dialysis unit.

9.3 Future Work

This thesis describes the acute and longer term consequences of AVF formation. However; there remain several unanswered questions.

Future studies are required to investigate longer term (> 3months) consequences of AVF formation. In addition, recording how the AVF haemodynamics change in response to starting dialysis and how the AVF flow affects systemic haemodynamics during dialysis is also an interesting area to study.

Furthermore, the effect of cardiovascular structure and function and different other haemodynamic variables on the function and survivability of AVF is another area for further study. In fact, such a study may yield data which could potentially enable intervention to improve longer term outcome of AVFs.

One of the limitations with our study was the small size of our cohort. A larger cohort could power the study enabling further statistical analysis to derive important information such as:

- Logistic regression model to investigate the factors affecting the success/failure of AVF operation.
- Bigger, more comprehensive regression model to look for the factors affecting the AVF flow and ΔPWV.
- Sub-classifying the cohort according to the anatomical location of the placed AVF and/or flow rate in the AVF.

With regards to the effects on AVF creation on microvasculature and endothelial function, a number of issues remain. Firstly, at this stage it is not clear what drives the acute changes observed. Although a model has been proposed, the pathophysiological mechanisms are still not clear. Measurement of markers of endothelial functions such as NO, ADMA and SDMA can assist in understanding these changes further. We are currently collaborating with certain centres in Europe to achieve this.

Secondly, it is unknown how the observed changes are maintained beyond the acute postoperative phase and if these changes affect the fistula survival and cardiovascular outcome in this group of patients. Certainly this is an area for further research in the future.

A longitudinal study to assess the effect of AVF flow on dialysis induced myocardial dysfunction is necessary to ascertain the findings in our cross sectional observation. In fact, our centre is currently running a study using cardiac magnetic resonance imaging to establish the longer term consequences of different variables on myocardial stunning and possible interventions to improve outcome in haemodialysis population.

Chapter 10

Abbreviations

10 Abbreviations

ACEi	Angiotensin-II converting enzyme inhibitor
Ach	Acetylcholine
ADMA	Asymmetrical dimethyl Arginine
AIx	Aortic augmentation index
ARB	Angiotensin receptor blocker
AVG	Arteriovenous grafts
AVF	Arteriovenous fistula
BP	Blood pressure
BIA	Bioimpedance analysis
BMI	Body mass index
BNP	Brain nitriuretic peptide
BRS	Baroreflex sensitivity
CKD	Chronic kidney diseases
CF-PWV	Carotid femoral pulse wave velocity
CI	Cardiac index
CO	Cardiac output
CRP	C-reactive protein
CrCl	Creatinine clearance
CVC	Central venous catheter
cTNT	Cardiac troponin-T
DBP	Diastolic blood pressure
ED	Endothelial dependant
EF	Ejection Fraction
eGFR	Estimated glomerular filtration rate
ESRF	End stage renal failure
Hcy	Homocystiene
IHD	Ischaemic heart disease
KDOQI	Kidney Disease Outcomes Quality Initiative

HDL	High density lipoprotein
HR	Heart rate
LDI	Laser Doppler imaging
LDL	Low density lipoprotein
LV	Left ventricle
LVH	Left ventricular hypertrophy
NED	Non endothelial dependant
NO	Nitric oxide
PWV	Pulse wave velocity
Qa	fistula blood flow rate
RIPC	Remote ischaemic preconditioning
RRT	Renal replacement therapy
SBP	Systolic blood pressure
SD	Standard deviation
SDMA	Symmetrical Dimethyl Arginine
SNP	Sodium nitroprusside
SV	Stroke volume
TBW	Total body water
TG	Triglyceride
TPR	Total peripheral resistance
UK	United Kingdom
US	United States
USRDS	United States Renal Data System
USS	Ultrasound
VC	Vascular calcification
VLDL	Very low density lipoprotein
WHO	World health organisation

Chapter 11

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